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NEUROGENIC FACTORS IN OVULATION

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Clinicians have often stated that the ovulatory mechanism in humans may be disrupted by neural disturbances as well as endocrine disorders, but there has been little experimental evidence to support this idea until recent years. In fact, most of the earlier experimental work indicated that the ovaries and the hypophysis are able to function in a normal manner in the absence of nervous connections. Greep (1936) found that the implantation of anterior pituitary grafts into the sella turcica of hypophysectomized rats induced regular estrous cycles, and in some cases these animals underwent complete reproductive cycles. Dempsey (1939) and Leininger and Ranson (1943) showed that normal cycles can occur in the guinea pig after transection of the pituitary stalk. Similar results were obtained in the rat by Dempsey and Searles (1943).

Ovulation is apparently not dependent upon the nerve supply to the ovaries since it may occur in ovarian grafts under the influence of the proper hypophyseal hormones.

Reynolds (1949) states that with few exceptions every reproductive and sex function about which we know can be subserved without the agency of the numerous and complex nervous connections of the uterus.

THE DEVELOPMENT OF THE CONCEPT OF NEUROHUMORAL CONTROL OF OVULATORY HORMONE RELEASE

Although Hinsey and Markee (1933) suggested that a neurohumoral substance involved in ovulation might intervene between the hypothalamus and the anterior lobe of the pituitary, this concept received little attention until 1946. In that year it was reported by Markee, Sawyer, and Hollinshead (1946) that localized bipolar stimulation of the hypothalamic region produced ovulation in 3 out of 4 rabbits. The same current was ineffective when applied directly to the hypophysis. It was concluded that ovulation was induced by the activation of a humoral pathway between the hypothalamus and the hypophysis. Subsequently, it was shown by Markee, Sawyer and Hollinshead (1948) that ovulation could be induced in the rabbit by the direct instillation of adrenalin, but not acetylcholine, into the hypophysis. These results indicated that the humoral link in the hypothalamic-pituitary path involved in ovulation is of an adrenergic nature. The same workers (Sawyer, Markee and Townsend 1949) subsequently discovered that the release of L. H. ("ovulatory hormone"), and consequently ovulation, could be blocked in the rabbit by the administration of either atropine or the adrenolytic drug Dibenamine immediately after coitus. These findings imply that the natural neurogenic stimulus includes a cholinergic as well as an adrenergic component, and it appears that the cholinergic

component precedes the adrenergic component since atropine must be given earlier than Dibenamine to block ovulation as effectively. Markee, Everett and Sawyer (1952) state that the natural neurogenic stimulus at copulation traverses the pituitary stalk, and the final pathway distal to the median eminence is probably the hypophyseal portal vascular system. Harris (1949) has shown that the return of normal sex function is closely correlated with regeneration of the portal vessels after pituitary-stalk-section in the female rat. It seems likely that the cholinergic component stimulates the secretion of the adrenergic mediator, which in turn stimulates the hypophyseal cells to release the luteinizing hormone.

These results were subsequently extended to a "spontaneously" ovulating species, the rat. Sawyer, Everett and Markee (1949) found that the ovulation which normally follows the injection of estrogen on the fourth day of pregnancy in the rat could be prevented by the injection of either atropine or Dibenamine. Everett, Sawyer and Markee (1949) and Everett and Sawyer (1949) have also shown that ovulation in the normal cyclic rat is prevented by the injection of either atropine or Dibenamine between 2:00 p.m. and 4:00 p.m. of the day of proestrus.

These reports establish the basis for a concept of neurohumoral control of the release of L. H. in both the rabbit and the rat which involves cholinergic and adrenergic components in sequential arrangement. With this background it is appropriate to examine similar experiments which have been conducted with large animals and the fowl. Subsequently, the nature of the neurohumoral mechanism and the relationship of the ovarian hormones to it will be considered in more detail.

EVIDENCE OF A NEURAL FACTOR IN THE OVULATORY MECHANISM OF CATTLE

One of the first indications that a neural factor might be involved in the ovulatory process in cattle is found in the report of Marion, Smith, Wiley and Barrett (1950). These workers studied the effect of sterile copulation on the time of ovulation in 25 dairy heifers. The heifers ovulated on an average 7.7 hours following the end of estrus when serviced by a vasectomized bull as compared to 9.9 hours when not serviced. The difference was significant.

Hansel and Trimberger (1951) compared the time of ovulation in dairy heifers in a control estrous period and in an estrous period in which atropine was administered at the beginning of heat. The results, summarized in Table 1, show that ovulation was delayed for considerable periods of time by the administration of atropine.

TABLE 1

The Effect of Atropine on Ovulation Time in Dairy Heifers

No. of Heifers	Control Period: Average Time from Beginning of Estrus To Ovulation (Hrs.)	Atropine-Treated Period*: Average Time from Beginning of Estrus To Ovulation (Hrs.)	Ovulation Delayed Hrs. (Range)
5	26	58	24 - 66

* Subcutaneously. Total doses ranged from 33 to 76 mg. atropine sulfate per kg. body weight.

In order to obtain some indication as to whether these results were due to atropine blockage of L. H. release from the anterior pituitary or to some non-specific effect of atropine, a second experiment was conducted in which the effect of the simultaneous administration of atropine and L. H. at the beginning of estrus was studied. The results are summarized in Table 2, and it may be seen that ovulation was not delayed when chorionic gonadotrophin (as a source of L. H.) was administered at the beginning of estrus along with atropine.

TABLE 2

The Effect of the Simultaneous Administration of Atropine and Chorionic Gonadotrophin at the Beginning of Estrus on Ovulation Time in Dairy Heifers

No. of Heifers	Control Period: Average Time from Beginning of Estrus to Ovulation (Hrs.)	Atropine*plus Chorionic Gonadotrophin**-Treated Period: Average Time from Beginning of Estrus to Ovulation (Hrs.)
5	26	28

*Subcutaneously. Doses ranged from 32 to 66 mg/kg. body wt.
**1000 I. U. intravenously and 2000-4000 I. U. subcutaneously.

Actually, ovulation occurred about 10 hours earlier than normal in 4 of the 5 heifers, while it did not occur until 72 hours from the beginning of estrus in the 5th heifer, which was given a lower initial dose of chorionic gonadotrophin. These results indicate that a neural factor having a cholinergic component is involved in the release of L. H. (or ovulating hormones) from the hypophysis and in ovulation in dairy cattle.

As a result of these experiments it seemed advisable to ascertain how the ovarian hormones would affect ovulation time when administered at the beginning of estrus. The administration of small amounts of progesterone at the beginning of estrus was found to significantly hasten ovulation (Hansel and Trimberger 1952), whereas estrogen administered at the beginning of estrus had no effect on ovulation time (Hansel, Trimberger and Bearden 1952). The results are summarized in Tables 3 and 4.

TABLE 3

The Effect of Progesterone Injected Subcutaneously at the Beginning of Estrus on Ovulation Time in Dairy Heifers

No. of Heifers	Av. Length of Estrus (Hrs.)	Av. Time from End of Estrus to Ovulation (Hrs.)	Av. Time from Beginning of Estrus to Ovulation (Hrs.)
CONTROL PERIOD			
11	18.6	12.3	31.0
PROGESTERONE-TREATED PERIOD (5-15 mg.)			
11	15.0	6.9	22.0

TABLE 4

The Effect of Estradiol Injected Subcutaneously at the Beginning of Estrus on Ovulation Time in Dairy Heifers

No. of Heifers	Av. Length of Estrus (Hrs.)	Av. Time from End of Estrus to Ovulation (Hrs.)	Av. Time from Beginning of Estrus to Ovulation (Hrs.)
CONTROL PERIOD			
11	19.6	10.9	30.0
ESTROGEN-TREATED PERIOD (1000 to 3000 I.U.)			
11	20.7	11.7	32.4

These results suggest that a pre-ovulatory rise in progesterone might be an integral part of the ovulatory mechanism in the cow. Edgar (1952), using a paper chromatography partition method, found that cow's follicular fluid near ovulation contained 3 μgm/ml. of progesterone; no progesterone was found in developing follicles, nor 3 and 4 days after heat in follicles which failed to ovulate. The graafian follicles of a sow nearly at ovulation contained 8 μgm/ml. of progesterone.

The relation of estrogen and progesterone to the neurohumoral mechanism for L. H. release will be discussed later.

EVIDENCE OF A NEURAL FACTOR IN THE OVULATORY MECHANISM OF SHEEP

Evidence for a neural factor in the ovulatory mechanism of the ewe has recently been obtained by Moore and Nalbandov (In Press).

These workers sutured plastic beads 8-11 mm. in diameter in one of the uterine horns of cyclic ewes 3 days after the first symptoms of heat. Distention of the uterine horn with plastic beads in this manner shortened the normal estrous cycle from an average of 16.3 days to an average of 11.9 days. One or more ovulations had accompanied heat in 18 of 22 ewes treated in this manner and slaughtered at various times after the onset of heat. In some instances follicles matured and ovulated as frequently as every 4 to 5 days. Removal of the beads from the uterine horn resulted in the resumption of cycles of normal length. This phenomenon appears to be of neurogenic origin, since cycles of normal length (16.1 days) occurred in ewes in which the section of the uterus containing the bead was denervated. Cycles of normal length also occurred when a section of the uterus was denervated without inserting a bead, when sham operations were made, and in control ewes which received no treatment.

EVIDENCE OF A NEURAL FACTOR IN THE OVULATORY MECHANISM OF THE FOWL

Huston and Nalbandov (1953) have found that placing a thread in the oviduct of laying hens completely suppresses ovulation in the great majority of the treated hens without causing regression of the ovary, comb or oviduct. The injection of either progesterone or a purified L. H. preparation

caused ovulation in hens with a thread in the oviduct as long as the injections were continued. It is thought that the presence of the thread prevents the secretion of amounts of L. H. sufficient to cause ovulation (ovulatory peaks). The presence of the thread does not appear to prevent the secretion of normal amounts of FSH since the ovary maintains its normal size for 25 days after the operation and contains follicles of ovulatory size in the same numbers as would be expected in normal hens for a similar period of time. These follicles must secrete estrogen since the normal size of the oviducts was maintained.

Van Tienhoven (1953) has found a regional difference in the sensitivity of the oviduct to this operation. A thread in the magnum was found to affect ovulation to a lesser extent than a thread in the isthmus, which blocks ovulation completely for at least 20 days. Threads in the fimbria and uterus produced intermediate results.

The authors feel that this neurogenic system may be of physiological importance in the normal hen as a timing device which prevents ovulation while a yolk (irritant) is passing through the oviduct.

THE RELATIONSHIP OF THE OVARIAN HORMONES TO THE NEUROHUMORAL MECHANISM FOR OVULATORY HORMONE RELEASE

From this brief discussion it may be seen that considerable evidence indicates that a neurohumoral mechanism for L. H. release exists in the rabbit, rat, cow, ewe and the fowl. The problem of integrating this knowledge into our previous concepts of the interrelations of the ovarian and hypophyseal hormones is a difficult one. Markee, Everett and Sawyer (1952) state that the ovarian steroid must "set the stage" in order for an effective nervous stimulus from the hypothalamus to occur in either the rabbit or rat. Sawyer, Everett and Markee (1949) found that the ovulation and luteal cholesterinization which normally follow the injection of estrogen on the 4th day of pregnancy in the rat could be prevented by the injection of either atropine or Dibenamine. Neither atropine nor Dibenamine prevented ovulation when L. H. was injected. These results indicate that the effect of estrogen in causing L. H. release in the pregnant rat is mediated, at least in part, by the nervous system. Everett (1948) showed that ovulation could be advanced by about 24 hours by the administration of progesterone to 5-day cyclic rats on the third day of diestrus. Later, Everett and Sawyer (1949) showed that this effect could be blocked by either atropine or Dibenamine. Sawyer, Everett and Markee (1950) produced ovulation without coital stimulation in the rabbit by treatment with estrogen and progesterone. Neither estrogen nor progesterone alone was effective. The experiments of Huston and Nalbandov (1953) in which progesterone injections overcame the blockage of ovulation caused by passing a thread through the oviduct have previously been cited. The ability of progesterone to stimulate ovulation under the proper conditions in many species is attested by numerous papers, in addition to those cited above. These papers have been reviewed by Hansel and Trimberger (1952) and by Markee (1951) and will not be discussed in detail.

It appears that the L. H. release mechanism is stimulated by the combined action of estrogen and progesterone, and since the effects of both

can be blocked by either Dibenamine or atropine, it is likely that their effects are mediated, at least to a large extent, through the hypothalamus. Markee, Everett and Sawyer (1952) have suggested that estrogen and progesterone facilitate L. H. release by lowering the threshold to extrinsic stimulation of a gonadotrophic sex center in the hypothalamus. It must be pointed out, however, that the possibility of a direct effect of the ovarian steroids on the hypophyseal cells still exists.

The ability of progesterone to hasten ovulation in the cow when given at the beginning of estrus and the failure of estrogen to do so indicates that progesterone action follows the action of estrogen in the normal sequence of events in this species. The fact that progesterone is found in the follicular fluid of the cow only near the time of ovulation lends support to this concept.

THE EXTEROCEPTIVE PATHWAYS AND THE EFFECTIVE STIMULI FOR THE NEUROHUMORAL MECHANISM OF OVULATORY HORMONE RELEASE

It is obvious that considerable species differences must exist as regards the stimuli necessary to activate the neurohumoral mechanism for release. In the rabbit the stimuli resulting from mating activate the mechanism, although in some cases ovulation can occur without actual mating. The stimulation of the hypophyseal cells appears to occur within a few minutes, since the blocking agents are ineffective if administered later than one minute after the end of coitus.

Everett and Sawyer (1949) (1950) have shown that the neurogenic stimulus for the ovulatory discharge of L. H. activates the hypophysis between 2 p.m. and 4 p.m. on the day of proestrus in their rat colony where the lights are on 14 hours each day. These workers also found that small doses of Nembutal and other central depressant barbiturates prevented ovulatory activation of the hypophysis when administered at 2 p.m. during proestrus. Persistence of graafian follicles for 2 to 3 days resulted when Nembutal treatment was repeated on successive afternoons at the same critical hour. If on any of these days treatment was omitted or postponed until after 4 p.m. pituitary activation occurred promptly and ovulation followed during the night. These results show that a 24-hour rhythm is a property of the L. H. release mechanism in these rats. Everett and Sawyer (1953) subsequently injected blocking doses of atropine at intervals during the 2-4 p.m. period of the day of proestrus and concluded from the results that the required duration of stimulation for the release of sufficient gonadotrophin for complete ovulation is about 20 to 35 minutes.

The experiments of Moore and Nalbandov (In Press) with the ewe, and Huston and Nalbandov (1953) with the fowl again illustrate species differences in the nature of the stimuli which are effective in activating the neurohumoral mechanism. In the fowl, an irritant in the oviduct blocks ovulation; in the ewe, uterine distention promotes ovulation. The afferent nerves of the organs concerned appear to mediate the stimuli involved in these experiments.

These results serve only to emphasize that the effective stimuli and exteroceptive pathways involved in the neurohumoral mechanism for L. H. release are as yet poorly understood in most species.

There are obviously many possible practical applications of knowledge

of the neurohumoral mechanism for ovulatory hormone release in improving the efficiency of reproduction in farm animals. In most cases, however, these practical applications must await the development of more exact knowledge of the nature of the neurogenic mechanism and the stimuli which activate it in each species.

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THE INFLUENCE OF THE ENVIRONMENT ON REPRODUCTION IN FEMALE FARM ANIMALS

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With the subdivision of the biological sciences into an ever increasing number of special areas, among the newer of which are radiobiology, bioengineering, biophysics, and microclimatology, biologists have tended to become more concerned with the parts of the organism rather than with the organism itself. The scientist of the first quarter of the present century was less interested in the broad effects of the environment upon man and beast than such early biologists as Lamarck and Darwin; his researches were concerned mainly with specific tissues, elements and molecules. At the midpoint of the twentieth century there seems to be a return of interest to the relationship of the individual to the environment. This stems from the fact that we know considerably more about the environment and because it now appears that we can expect to exert considerable influence upon it through technological means.

An analysis of the influence of the environment on reproduction cannot be limited to the reproductive organs alone. In its broadest sense the environment includes and affects all things physical and philosophical. Scientists, farmers and the average citizen find themselves using such terms as nutritional environment, climatic environment, and social and political environment. Even the latter has a profound effect on all of agriculture including reproduction. Decisions as to whether we shall produce eggs (white or brown), milk, cream, or butter are most certainly reproductive problems tempered by political and economic influences.

The writer neither proposes to confine his discussion exclusively to reproduction in female farm animals nor to present a complete literature review of the subject. The literature cited does not necessarily indicate the priority of the investigator.

The influence of length of day on growth and reproduction of plants was first recognized by Klebs (1918). The term photoperiodism was introduced by Garner and Allard (1920) to describe the developmental responses of plants to length of day. Numerous books and reviews have been published on this topic and are cited in a recent review by Leopold (1951). Since flower initiation is one of the most dramatic photoperiodic changes, and since this is a reproductive process, it seems appropriate to make at least passing mention of it. It seems clear that the metabolic changes initiated in plants by light have many similarities to those which can be induced in animals. In both there are measurable changes in ion uptake and transfer, and in both hormonal activity is affected.

AGRICULTURAL PRODUCTION AND CLIMATE

The absolute dependence of plants on proper climatic conditions is so complete that farming is largely a seasonal operation. The climatic forces in operation on farms affect soil physics and chemistry, crop yields and composition and virtually every economic function of animal production. As a result of the seasonal or day to day changes in plants, temperature, light, humidity, and even cosmic rays, the farm animal finds itself subjected to an incomprehensible number of variables. In a sense, this situation may be similar to constantly changing the fuel of a high compression engine. There is considerable difference in the combustion properties of high octane gas and whale oil, although both are fuels. There is just as much difference to the animal between a lush legume pasture and timothy hay. The engine and the animal both exhibit suboptimum performance if the environment is not optimum.

It has long been known that both milk and butterfat production decline during the late summer in the European breeds of dairy cattle common in the United States. It has been assumed that the summer depression of lactation was the result of high temperatures and the reduction in both quantity and quality of herbage under the average farm conditions. It has likewise been recognized that European breeds of cattle are poorly adapted to tropical conditions as compared with some breeds of Indian cattle. Brody and co-workers (1948-53) have conducted extensive studies on the effects of the environment on lactation and numerous other physiological processes of dairy cattle. In brief, milk production and TDN consumption fall rapidly at temperatures above 85° F. Rectal temperature, pulmonary ventilation, respiratory vaporization, and respiratory rate rise markedly above 85° F. The most pronounced blood changes were the increase of creatinine and the reduction of cholesterol with increasing temperature. It seems clear that the entire metabolic pattern of cattle, including endocrine activity, may be altered by a changing environment. This in turn would seem to at least have a bearing on reproductive efficiency in cattle.

It is likewise well known that egg numbers, egg weights and fertility are minimum during the summer months. This has a profound effect on the economics of poultry production. In spite of constant selection for high egg production and the elimination of seasonal trends, eggs are in the greatest supply and at the lowest price during the spring, the natural reproductive period of North American poultry and birds.

Most breeds of sheep kept in the northern hemisphere are strictly seasonal breeders. This imposes very practical limitations in the production of sheep for meat purposes. Not only are the pounds of meat per female per year limited, but the supply of lamb meat fluctuates widely throughout the year. Thus far the efforts of the geneticist and the physiologist to solve this problem have not been too rewarding.

The two litter a year system of swine production has become so well established in the central states that the desirability of other systems of production have only recently been investigated. It has commonly been assumed that swine are capable of efficient reproduction throughout the year and that the practice of spring and fall breeding has been a matter of choice of the producer. This assumption may not be true. Studies of the

effects of climate on the growth and feed efficiency of swine suggest that this species may be the most sensitive of all the farm animals to temperature change. Heitman and Hughes (1949) showed that in hogs weighing from 70 to 144 pounds growth rate and feed efficiency were maximum at an environmental temperature of 75° F. In heavier hogs the optimum temperature was approximately 60° F. In pregnant sows respiratory rate increased markedly at ambient temperatures above 88° F. and body temperature rose rapidly when the environmental temperature was about 94° F. (Heitman, Hughes, and Kelly, 1951). The California results suggest that heat prostration and death of the pregnant sow will probably precede fetal death and abortion.

It is not uncommon under extreme summer conditions to observe lactation failure in swine showing evidences of heat prostration.

It seems clear therefore, that the environment exercises an important effect on such important economic and physiologic functions as reproduction, lactation, and egg production.

THE INFLUENCE OF LIGHT

In view of the extraordinary influence of light on plants it is surprising that photoperiodicity was not discovered earlier than 1918. The association of seasonal variations in amount of light and egg production of the domestic chicken by Lewis et al. in 1919 was a noteworthy discovery, since the effects of light are not so obvious in animals.

Rowan's studies on the migration phenomena in the Junco (1925-29) and those of Bissonnette (1930-31) with the European starling have become biological classics. These investigators showed that progressive illumination during the winter induced testis activity and spermatogenesis and that a diminution in light was followed by testicular involution.

The use of artificial light to alter the normal patterns of egg production in the chicken and turkey is an established poultry practice. It is the consensus of opinion that in the chicken seasonal trends in egg production, rather than increase in total annual egg production, is affected. Ogle and Lamoreux (1942), for example, showed that the use of morning lights during the fall to increase day length stimulated fall and winter egg production. Spring egg production in lighted flocks was lower than the normal spring production of nonlighted birds.

Bissonnette's discovery (1932) that artificial light would induce estrus in the ferret stimulated interest in the sexual photoperiodicity of mammals. It had been recognized that most wild mammals such as the field mouse, raccoon, and ground squirrel were long-day seasonal breeders and that the sheep, goat, and deer breed during the fall or short-day season.

During the past decade several significant researches have been carried out with goats and sheep. Bissonnette (1941) reported that reversal of the seasonal cycle of day length leads to partial reversal of the sexual cycle of Toggenburg and Nubian goats. Increasing daily light periods from January 25 to April 5, followed by decreasing light until July 5, resulted in the cessation of estrual cycles in February instead of March and the resumption of estrus in May and June instead of September.

Marshall (1937), following his observation that the transportation of sheep and deer from one hemisphere to another caused a reversal of the

breeding season, concluded that these animals react to light, particularly the diminution of light. Sykes and Cole (1944) subjected eight ewes to a regime of gradually increasing light during March followed by a reduction of light during April and early May. On May 6 the sheep were receiving 6 hours of light less per day than was available in the normal environment. It was tentatively, but correctly, concluded that the alteration in amount of light was responsible for the re-establishment of the sexual cycle of anestrous ewes and the subsequent birth of 5 lambs.

The researches of Yeates (1949) and Hart (1950) appear to dispel any doubts that most breeds of sheep exhibit sexual photoperiodicity. The broad pattern of the photoperiodic phenomenon has been established, but many questions remain unanswered. Past research workers have given very plausible explanations for the seasonal sexual behavior of sheep. At one time it appeared that variations in plane of nutrition were the chief regulative factor; it later seemed that the seasonal temperature changes were most important. It seems appropriate, therefore, to recognize the complexity of all the factors which compose the environment.

Yeates (1949) methodology involved changes in amount of light at weekly intervals in an attempt to duplicate the seasonal changes which occur in nature. The ewes were Suffolk X Border Leicester-Cheviots. Incandescent light amounting to 11 f.c. in the center of the pen was supplied by 100 w. bulbs. During the day the sheep were turned out to graze and were given supplementary hay, grain and roots to maintain them in satisfactory condition. Yeates concluded that seasonal changes in length of day were responsible for the reproductive behavior of the grade Suffolk ewes in his experiments and that "this finding applies generally to seasonal breeding sheep in all regions." The onset of the sexual season occurred 13-16 weeks after the change from increasing to decreasing length of day. The cessation of the sexual season occurred 14-19 weeks after the change from decreasing to increasing length of day. Yeates (1949) theorizes that "sheep show a gradation in length of sexual season according, in general, to the latitude of their place of origin." He also concludes that seasonal variation in fertility of rams appears to be controlled by the light environment.

Hart (1950), using the same flock studied by Yeates, investigated the effects of a fixed dark-light rhythm without the necessity of gradually decreasing the amount of light.

Light was supplied by 80 w. daylight fluorescent bulbs. The intensity beneath the bulbs was 30 f.c. and 25 f.c. within one foot of the walls. The light schedules included systems such as 4 hour light, 2 hour dark, 4 hour light, 14 hour dark; and 4 hour light, 8 hour dark, 4 hour light, 8 hour dark. No treated animal failed to come in heat. In virgin ewes estrus was induced 3-4 weeks after the beginning of treatment and at 91 days in suckling ewes. Hart concluded that gradual changes in light are not essential. A ratio of 1 part of light to 2 parts or more of dark were effective in inducing estrus.

Hafez (1950) classified British sheep into 3 groups. The Blackface Mountain, Border Leicester and Welsh Mountain (Scottish or Welsh breeds) bred when length of day was 11½-12½ hours or less. The Romney Marsh and Suffolk (southern England) bred when the length of day was 12-13½ hours or less. The Dorset, which presumably carries Spanish Merino blood, bred when the hours of daylight were 12-17 or less.

THE INFLUENCE OF TEMPERATURE

In mammals, including man, it is commonly believed that high environmental temperatures are deleterious to both physiological and mental activity. The desire of man to remain cool during the summer months has a profound effect on his entire economy. In some regions of the world the scene of government shifts from winter to summer headquarters and only essential business is transacted. In recent years the trend toward complete summer air conditioning has reached the point where it is regarded as mildly pathological to work under normal summer temperature conditions. From the standpoint of agricultural research workers this trend is rapidly resulting in the air conditioning of all animal rooms but, unfortunately, not many class or office rooms.

Although there is little doubt that the summer reproductive efficiency of the chicken, rat, mouse, and guinea pig is low, and that the fertility of man and of cattle in some portions of the world is minimum in summer, there are few quantitative data on the specific effects of temperature on ovarian activity.

The assumed importance of temperature probably stems from the many researches on the sensitivity of the mammalian testis to abdominal temperature conditions. The early studies on the cryptorchid testis reviewed by Moore (1932) clearly showed that the scrotum performs a vital temperature regulating function. The natural occurrence of cryptorchidism, the experimental replacement of the testes in the abdominal cavity, and the artificial induction of increased testis temperature, produce immediate damage to the proliferating germinal epithelium. Studies by McKenzie and Berliner in 1937 with rams, Erb, Andrews, and Hilton in 1942 with the dairy bull, and by numerous other workers subsequently, indicate that when high summer temperature conditions prevail, semen quality is reduced. Casady, Myers, and Legates (1953), employing a controlled environmental chamber, concluded that "under chamber conditions spermatogenesis in the young dairy bull may be impaired when the animal is continuously exposed to temperatures exceeding 85° F. for periods exceeding five weeks."

As reviewed by Erb and Waldo (1952), the available evidence still suggests that, in areas where high environmental temperatures are common during a portion of the year, breeding efficiency in dairy cattle does decline during the summer. A series of studies by Mercier and Salisbury (1946-47) in eastern Canada and New York State seem to prove that hours of daylight rather than temperature were the major variable. Erb and Waldo (1952) found that in northwestern Washington the results were very similar to those reported by Mercier and Salisbury (1946-47). The fertility of dairy cows, as measured by over 90,000 first and second artificial inseminations, was lowest in January and highest in September.

Experimental evidence of a direct relationship between temperature and fertility in the mare, sow, and ewe is largely lacking. Studies being made with these animals from the standpoint of general heat tolerance do indicate, however, that temperature does have a marked effect on their physiology. Rising temperatures in general reduce feed intake, growth rate and milk production. They are accompanied by increased respiratory activity and numerous vascular changes. Until the temperature regulating

devices fail at high temperatures, there is a decrease in rate of metabolism. Following the inability of the animal to maintain normal temperature, body temperature rises, and heat prostration and death may follow. Because of the alteration of so many physiological processes the circumstantial evidence suggests that the reproductive processes might well be affected.

THE INFLUENCE OF IRRADIATION

Existence in the Atomic Age warrants a passing mention of the effects of irradiation on reproduction. It has been known since the turn of the century that X-irradiation produces severe ovarian damage. It is now known that, depending on dosage, X-rays may produce varying degrees of ovarian damage, including permanent sterility (Warren, 1944). Much work is in progress on the effects of gamma and beta rays, fast and slow neutrons and the radiations of numerous isotopes which emit alpha, beta, and gamma rays. A review of the effects of irradiation on the ovary (Bloom, 1948) indicates that X-rays, gamma and beta rays, and fast and slow neutrons produce varying degrees of ovarian damage depending on dosage-time relationships.

It is not within the scope of this paper to consider the role of irradiation in genetic variation, but it is clear that such variation would be mediated through effects on the germ cells.

THE MODE OF ACTION OF EXTERNAL ENVIRONMENTAL FACTORS

Certain parts of the organism, especially the skin and the eyes, may be directly affected by the environment. The thermo-regulatory function of the scrotum is likewise directly responsive to environmental temperature. It is at least theoretically possible that under extreme temperature conditions heat damage to the germinal epithelium of the testis might result. It is extremely doubtful if the ovary would be directly affected by any environmental factors except radiation from X-rays, neutrons, beta, or gamma rays.

In seasonal breeders a preponderence of evidence seems to indicate that the reproductive cycle is regulated by those environmental factors which increase or inhibit the production of gonadotropins by the anterior pituitary gland.

Benoit's (1936-44) researches on the duck show that increasing amounts of light increase pituitary gonadotropin secretion. Light may produce its effects through the eye, but light may stimulate the pituitary through penetration of head tissues other than the eye. His work showed differences due to light intensity and wave length. Red light was effective but blue-violet rays were effective only when falling directly on the exposed anterior pituitary gland. The ferret (Bissonnette, 1936) does not respond to light in the absence of the eye. The ferret may exhibit reproductive activity in the absence of the eye but seasonal changes do not occur. It has been assumed that the pituitary of the ferret is situated too deeply to be directly affected by light. If this assumption is correct, it is probable that farm mammals which exhibit sexual photoperiodicity must be stimulated

through the eye and other neural pathways rather than by the effects of light directly on the pituitary.

There is an extensive literature on the effects of intensity and wave length of light. Since it involves chiefly birds and the ferret, the reader is referred to a review paper of Yeates (1949).

The mechanisms by which changes in environmental temperature affect gonad function are obscure. The effects of temperature on the intact organism are not so obscure. In general, in homothermic animals, decreasing the environmental temperatures below the critical temperature increases oxygen intake, nitrogen excretion, and food intake; both thyroid and adrenal cortical activity are increased. Raising temperatures above the critical temperature brings into play all the physiological mechanisms concerned with heat loss. Not only is heat loss facilitated by radiation, conduction, and the vaporization of water, but heat production is reduced. The latter is accomplished through decreased feed intake, reduced body activity, and reduced metabolic activity. Most reproductive physiologists have explained the reduction in summer reproductive activity as one of the end results of decreased metabolism.

Berliner and Warbritton (1937) hypothesized that the seasonal decline in fertility of the ram was due to decreased thyroid activity. Bogart and Mayer (1946) found that in rams the administration of thyroxine improved fertility at high environmental temperatures. In contrast, Yeates (1949) concludes that seasonal changes in fertility of the ram appear to be regulated through photoperiodic mechanisms.

SUMMARY

The climatic forces in operation on farms affect soil physics and chemistry, crop yields and composition, and virtually every economic function of animal production.

Among the important economic and physiologic functions of farm animals affected by seasonal climatic changes are milk and butterfat production; the quantity, quality, fertility, and hatchability of eggs; the level of fertility in most farm animals and limited seasonal reproduction in sheep; and the rate of growth and efficiency of feed utilization in all meat animals, especially swine.

The most important known climatic factors which influence reproduction and reproductive efficiency are light and temperature.

The natural or artificial increase in length of day stimulates egg production in chickens and turkeys and a wide variety of wild birds. It appears that increased ovarian activity is brought about by increased anterior pituitary gonadotropin secretion. Light is known to be effective through the eye and probably some other neural pathways.

The natural or artificial decrease in length of day has been shown to induce ovarian activity and the establishment of the sexual cycle in anestrual sheep and goats.

The role of environmental temperature in seasonal changes in fertility in cattle, sheep, swine, chickens, and man is incompletely known. In regions where prolonged high temperatures persist it appears that the level of fertility declines in July and August in the northern hemisphere. Since maximum temperatures and maximum day length coincide, the

separation of these two factors under normal conditions of livestock management is not possible. The evidence available from several climatic laboratories clearly shows that rising temperatures affect a variety of physiologic functions such as respiration, circulation, a number of specific metabolic and endocrine functions, and lactation. Whether the reproductive functions are affected directly through the anterior pituitary gland or secondarily is not definitely known.

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RELATION OF NUTRITION TO ENDOCRINE-REPRODUCTIVE FUNCTIONS[1]

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A great mass of data has been accumulated during the past 35 years showing that underfeeding or deficiencies of certain vitamins can interfere with reproductive functions in all mammalian species studied. Although this information has indicated which dietary factors may be important in reproduction, there was until recently little understanding of the actual mechanisms involved. Reproduction is primarily controlled by the endocrine system, and particularly by the gonadotropic and gonadal hormones. It is logical to assume therefore, that many if not most of the actions of nutrition on reproduction are mediated through altering (1) hormone production by the endocrine glands,(2) tissue reactivity to hormone stimulation,(3) the metabolism of hormones. Not only may nutrients influence hormonal functions, but the reverse may be true as well. Hormones normally help regulate the metabolism of protein, fat, carbohydrate, and vitamins. Recent studies in our and other laboratories have indicated that endocrine imbalances, brought about as the result of an excess or deficiency of a particular hormone in the body, may increase requirements for dietary factors necessary for growth and reproduction. Many other types of stresses, such as disease, toxins, physical exertions, etc., may also increase nutritional requirements for growth and reproduction.

This paper will deal primarily with the physiological or endocrine aspects of nutritional-reproductive interactions, as indicated above. Female reproduction will be emphasized, since this is the subject of the present research conference. It will be understood, however, that the male reproductive system is similarly influenced by nutrients in most instances.

Excellent reviews of the relation of nutrition to reproduction in farm animals have been prepared by Friedman and Turner (1939), Guilbert (1942), Reid (1949), and Asdell (1949). Similar reviews dealing mostly with laboratory animals and other species have appeared by Mason (1939), Herts (1946), and Russel (1948). Discussions of general hormonal-nutritional interrelationships have been written by Samuels (1948) and

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Ershoff (1952). An intensive coverage of this entire subject cannot be attempted here, but it is hoped that it will be sufficient to highlight some of the more significant aspects of this important work.

EFFECTS OF NUTRITION ON GENERAL REPRODUCTIVE FUNCTION

Underfeeding or starvation

Reduced food intake has been shown to delay sexual maturity and inhibit estrous cycles in all mammalian species studied. Evans and Bishop (1922) demonstrated that in the immature female rat underfeeding results in small ovaries, absence of large follicles, follicular atresia, and failure of ovulation. They were able to completely suppress estrus in rats for as long as 375 days by underfeeding. Inanition in adult animals results in failure of follicles to develop to maturity, follicular atresia, and loss of libido (Evans and Bishop, 1922; Loeb, 1921; Marrian and Parkes, 1929). Restricted food intake may induce resorption or abortion of the embryo during the first half of gestation, but starvation after this period does not necessarily interrupt gestation although it may result in stillbirth or delivery of runty offspring (Barry, 1920. Cited by Mason, 1939). This indicates that adequate caloric intake by the mother is more critical for the fetus during the first half of pregnancy, since after this period the nutritive needs of the fetus can be more easily met by drawing from maternal tissues.

Protein, fat, and carbohydrate deficiencies

Both the quantity and quality of protein may be important for normal reproductive function (Guilbert and Goss, 1932; Pearson, Hart, and Bohstedt, 1937). Guilbert and Goss (1932) claimed that the minimal protein level in the diet necessary to maintain estrus in rats was approximately 7 per cent. Impaired gonadal function has also been observed on rations deficient in phenylalanine, lysine, leucine, histidine, and tryptophane (see Ershoff, 1952). In the rabbit, however, protein requirements may not be so critical, since it was found that 2-percent-protein diets and protein-free diets, fed for relatively long periods, did not interfere with estrus and ovulation despite a 25 per cent loss in initial body weight (cited in Friedman and Turner, 1939). It should be kept in mind that most of these earlier studies dealing with the relation of protein to reproduction were complicated by various vitamin deficiencies (including vitamin B_{12}) and inanition, and the problem deserves further study.

Deficiencies of unsaturated fatty acids have been reported to result in cessation of estrous cycles and ovulation in rats (Burr and Burr, 1930; Evans, Lepkovsky, and Murphy, 1934). However, inanition and loss of body weight have not been ruled out as contributing factors. It is generally agreed that carbohydrates as such are not essential for reproduction.

Vitamin and mineral deficiencies

In general, deficiencies of B-vitamins have been shown to elicit the

same inhibitory effects on reproduction as reduced food intake or starvation. This is not surprising since deficiencies of B-vitamins are usually accompanied by reduced appetite. Delayed sexual maturity and atrophy of the ovaries and other sex organs have been demonstrated in animals deficient in thiamine, riboflavin, pyridoxine, pantothenic acid, biotin, and vitamin B_{12} (see Ershoff, 1952). Other workers have shown that, even on diets abundantly supplied with all the known B-vitamins, reduced caloric intake resulted in cessation of estrous cycles (Mason, 1939).

A deficiency of vitamin C apparently does not interfere with estrus or ovulation in guinea pigs with scurvy (Goettsch, 1930). Mason (1939) also states that no specific reproductive disturbances have been found in man, monkey, and guinea pigs with scurvy. Vitamin E deficiency does not hinder normal estrus, ovulation, and implantation in the rat; during pregnancy, however, resorption of the fetuses occurs (Evans and Bishop, 1922; Evans and Burr, 1927). A lack of vitamin A results in keratinization of epithelial cells in all parts of the body, including the uterus and vagina, but there is no interference with the estrous cycle and conception is possible. During the latter part of gestation, however, there is abortion or birth of weak or dead offspring (Evans and Bishop, 1922; Mason, 1935). In vitamin E deficiency, the mesodermal and hematopoetic tissues are primarily affected, whereas in vitamin A deficiency, the epithelial tissues are mainly attacked.

It is generally agreed that an uncomplicated deficiency of vitamin D does not interfere with reproductive activities in mammals. There is also no convincing evidence that any mineral, including phosphorus, calcium, or manganese, is needed for normal reproduction (see Mason, 1939; Friedman and Turner, 1939). When such claims have been made, particularly in respect to a deficiency of phosphorus, they have been complicated by low protein intake and inanition.

It should be emphasized that almost all dietary factors have been claimed to be essential for reproduction at one time or another (Friedman and Turner, 1939), but only few have conclusively been shown to be needed thus far. More critical research, dealing with the specific effects of single deficiencies on endocrine and reproductive organs, will be required to properly evaluate the need for particular nutrients for reproduction.

EFFECTS OF NUTRITION ON HORMONE SECRETION

In general, underfeeding or B-vitamin deficiencies reduce the secretory activity of all endocrine glands, with the exception that during severe starvation the adrenal glands increase in size and function. The typical effects of underfeeding on the weight of endocrine glands of rats are shown in Table 1. Mature female rats of the Sherman strain were placed on, ad libitum, 3/4, 1/2, 1/4, and no-food regimens. The food consumption of the 3 partially-fed groups was determined on the basis of the daily food intake of the ad libitum fed group. The unfed group was sacrificed at the end of 7 days and the others at the end of 14 days. It can be seen that the weights of the pituitary, thyroid, adrenals, and ovaries were decreased in all the underfed groups, with the exception of the adrenals of the completely starved group.

TABLE 1

WEIGHTS OF ENDOCRINE GLANDS OF INTACT RATS ON RESTRICTED FOOD INTAKES *

No Rats per Group	Treatment	Original Body Weight (gm.)	Final Body Weight (gm.)	Per cent Diff. in Body Weight	Ovarian Weight per Rat (mg.)	Uterine Weight per Rat (mg.)	Adrenal Weight per Rat (mg.)	Thyroid Weight per Rat (mg.)	Pituitary Weight per Rat (mg.)
10	Controls fed ad lib.	196	220	+12	62.4	393.4	63.2±1.8**	18.1±0.9**	15.2±1.2**
10	Fed 3/4 ad lib.	193	197	+ 2	52.3	374.5	54.0±2.5	15.9± 0.9	11.7± 0.6
10	Fed 1/2 ad lib.	197	173	-12	50.5	256.0	47.3±2.0	12.6±0.6	10.9±0.4
9	Fed 1/4 ad lib.	200	153	-24	46.7	154.3	47.7±3.0	13.0±0.5	9.1±0.2
9	No food	209	141	-33	51.1	184.0	61.8±3.6	10.6±0.2	8.9±0.4

* From Meites and Reed (1949)

** Standard error of the mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

TABLE 2

ASSAYS OF THE GONADOTROPHIN CONTENT OF PITUITARIES OF INTACT AND OVARIECTOMIZED RATS ON RESTRICTED FOOD INTAKES *

Donor Rats			Recipient Rats ** (Gonadotrophin Assays)			
			Ovarian Wt. Per		Uterine Wt. Per	
Procedure	Av. Pit. Wt. (mg.)	Body Wt. (gm.)	Rat (mg.)	100 gm. Body Wt. (mg.)	Rat (mg.)	100 gm. Body Wt. (mg.)
Intact						
Controls, fed ad lib.	15.2	77	18.6± 2.3 †	24.2	53.3± 2.5 †	69.5
Fed 3/4 " "	11.7	77	19.2± 2.5	25.0	71.9± 12.0	93.8
" 1/2 " "	10.9	75	17.8± 2.2	23.8	72.4± 9.2	87.0
" 1/4 " "	9.1	78	20.0± 2.3	22.7	88.6± 3.9	92.5
No Feed	8.9	81	17.8± 2.4	24.8	65.0± 9.1	110.0
Controls uninjected		74	15.2± 1.8	20.5	23.4± 4.6	31.0
Ovariectomized						
Controls, fed ad lib.	11.3	68	65.7± 6.2	96.3	84.4± 5.0	123.8
Fed 3/4 " "	13.2	74	63.5± 11.8	85.8	116.0± 7.0	158.0
" 1/2 " "	11.8	71	59.6± 7.2	84.1	85.0± 10.0	120.0
" 1/4 " "	11.1	73	65.6± 5.5	90.1	99.4± 5.4	136.5
No Feed	11.7	72	65.7± 6.2	96.3	84.4± 5.0	123.8

* From Meites and Reed (1949).

** The equivalent of one donor rat pituitary was injected into each recipient rat.

† Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

Anterior pituitary function

There is convincing evidence that inanition or B-vitamin deficiencies can reduce the secretion of gonadotropic and other hormones by the anterior pituitary. Evans and Bishop (1922), Marrian and Parkes (1929), and Stephens and Allen (1941) showed that the ovaries of underfed rats and guinea pigs are responsive to injections of gonadotropic hormones, indicating that the effects are on the pituitary and were not due to decreased sensitivity of the ovaries. Mulinos and Pomerantz (1940, 1941) termed the condition associated with inanition "pseudohypophysectomy" because it resembled the condition which normally follows surgical removal of the pituitary gland.

Although it is agreed that gonadotropic activity by the anterior pituitary is reduced during inanition, there is some question as to whether the actual content in the pituitary is reduced, unchanged, or increased. Mason and Wolfe (1930), Werner (1939), and Guilbert and Goss (1932) reported a decrease, but Marrian and Parkes (1929), Maddock et al. (1947), and Meites and Reed (1949) found either no change or an increase in content. Some of the data from the latters' experiment is shown in Table 2. Gonadotropic potency was measured by injecting the equivalent of 5 pituitaries from each group into 5 immature female rats during a 5-day period. On the sixth day the rats were sacrificed and the ovaries and uteri were weighed. It can be seen that neither in the intact nor in the ovariectomized rats on restricted food allowances was the gonadotropic content of the pituitary reduced below that of the controls fed ad libitum. It is believed that this represents a failure of secretion and release of gonadotropic hormones by the pituitary during inanition.

Several investigators have reported that diets deficient in protein impair gonadotropic secretion by the pituitary (Courrier and Raynaud, 1932; Guilbert and Goss, 1932; Samuels, 1948). It has been claimed that these effects were not due to decreased food intake (Samuels, 1948).

Deficiencies of vitamin A (Sutton and Brief, 1939; Hodgson et al., 1946; Erb et al., 1947) or E (Biddulph and Meyer, 1941) were reported to produce morphological changes in the pituitary characteristic of reduced function. This work was based on the premise that pituitary changes might account for the reproductive disorders observed in animals deficient in these factors.

Despite the above reports, and others indicating changes in the gonadotropic content of the pituitary, it appears doubtful that lack of viatmin A or E, exclusive of effects due to inanition or debility, seriously hinders the production of gonadotropic hormones, or that gonadotropic hormones can alleviate the injurious effects caused by deficiencies of these vitamins. Mason (1933, 1935) reported that vitamin A could not restore the sex glands of rats on reduced caloric intake. He also noted that gonadotropic injections into vitamin A- or E-deficient male rats did not improve spermatogenesis and even aggravated it. Neither pituitary tissue, chorionic gonadotrophin, progesterone, estrone, or prolactin were effective in maintaining gestation in vitamin E-deficient rats (see Ershoff, 1952). In deficiencies of vitamin A or E therefore, we have two examples of effects of nutrients on reproduction which are not mediated primarily through the endocrine system.

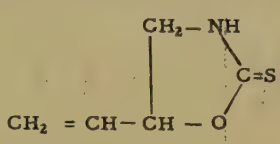
Thyroid function

The thyroid profoundly influences reproductive activities, as indicated elsewhere in this symposium (see Reineke). It is therefore necessary to consider some of the nutritional factors which can alter thyroid function, since this in turn will affect reproductive performance.

The effects of undernutrition on thyroid activity were studied in our laboratory in fifty young female rats of the Sherman strain. These rats were divided into five equal groups and were underfed according to the same scheme outlined on page 21. Eight hours prior to sacrifice, each rat was injected intraperitoneally with 0.2 ml. of carrier-free I^{131} (radioactive) containing approximately 2 microcuries of activity. This served as an indicator of thyroid function. It can be seen (Table 3) that thyroid weight was reduced in all the underfed group, the degree depending on the severity of underfeeding. The amount of I^{131} taken up by the thyroids was also reduced in all the underfed groups, again depending on the severity of food restriction.

There is no need to consider the well established effects of iodine deficiency in producing goiter and hypothyroidism. However, the presence of goitrogenic substances in numerous foods has only recently been demonstrated. The family Brassicaceae, which includes cabbage, brussels sprouts, cauliflower, rutabaga, turnip, rape, and kale, has been found to be particularly active in this respect. Moderate goitrogenic properties were also demonstrated in peach, pear, strawberry, spinach, and carrot, when assayed in man (Greer and Astwood, 1948).

A potent goitrogenic substance which was first isolated from rutabaga and has since been found in other members of the family Brassicaceae, is L-5-vinyl-2-thiooxazolidone. This substance, the formula for which is given below, is believed to be the chief or possibly the only goitrogen in the Brassicaceae (Greer, 1950).



The potency of this thionamide has been found to be the same as that of thiouracil in man and is 1/5 as potent as thiouracil in the rat (Greer, 1950). It is more concentrated in seeds than in the root, stem, or leaves. If the whole seeds or roots are boiled, baked, or steamed, the activity is destroyed. This substance, like thiouracil, acts by interfering with the synthesis of thyroid hormone.

It has been claimed that soybeans are goitrogenic in rats, but apparently this is due to low iodine in the diets used (Halverson, Zepplin, and Hart, 1949). Cyanides have been demonstrated to produce goiter, but this can also be overcome by adding iodine to the ration (Greer, 1950). These substances, therefore, differ in their actions from the Brassicaceae since goitrogenicity in the latter cannot be overcome by supplementing the diet with iodine. Although it does not appear very probable, the possibility

TABLE 3

EFFECTS OF UNDERFEEDING ON UPTAKE OF I^{131} BY THE THYROIDS OF RATS*

Treatment	Orig. No. per Group	Final No. per Group	Av. Orig. Body Wt. (gm.)	Av. Final Body Wt. (gm.)	Av. Thyroid Wt. (mg.)	Av. Uptake of I^{131} per Thyroid (per cent)	Av. No. Counts per Thyroid
Controls, fed ad lib.	10	10	147.0	169.5	13.79	7.9	20.05±1.52**
Fed 3/4 ad lib.	10	10	146.6	148.5	10.34	6.1	16.71±1.64
" 1/2 " "	10	10	145.5	126.6	8.97	5.0	13.26±1.32
" 1/4 " "	10	8	145.4	111.0	8.60	4.1	10.51±1.00
No feed	10	4	146.0	87.0	7.20	3.2	8.92±0.39

* From Meites and Wolterink (1950)

** Standard error of mean.

TABLE 4

EFFECTS OF REDUCED CALORIC OR PROTEIN INTAKE ON SEMINAL VESICLE RESPONSE TO P.M.S. IN MICHIGAN STATE COLLEGE RATS

Group	No. of Rats	Diet Fed	Given P.M.S.	Average Initial Body Weight (gm.)	Average Final Body Weight (gm.)	Average Seminal Vesicle Weight (mg.)	Average Seminal Vesicle Wt. per 100 gm. Body Weight (mg.)
1	7	Purina chow	No	43.7	168.0	81.6	48.1± 3.4 *
2	7	" "	Yes	43.9	151.0	160.9	110.0± 13.4
3	5	3/4 " "	"	43.2	127.0	125.6	98.8± 17.2
4	6	1/2 " "	"	43.9	84.9	91.7	107.3± 6.2
5	7	Semi-synthetic	No	43.0	156.0	65.5	41.6± 2.0
6	6	"	Yes	43.0	154.0	156.4	99.2± 12.0
7	5	3/4 "	"	43.2	113.0	102.3	90.1± 10.9
8	7	1/2 "	"	43.4	89.7	91.5	102.6± 12.4
9	8	15 % Casein	"	43.7	134.9	138.1	103.1± 12.9
10	7	10 % "	"	43.7	86.3	87.6	95.4± 14.4

* Standard error of mean.

must be considered that animals may eat sufficient quantities of these goitrogen-containing foods to develop symptoms of hypothyroidism.

EFFECTS OF NUTRITION ON TISSUE REACTIVITY TO HORMONES

Gonadotropic hormones

It has already been indicated that the gonads of underfed or B-vitamin deficient animals remain responsive to gonadotropic hormones. The following study was undertaken in our laboratory (Yadu, 1950), to determine the quantitative effects of a constant dose of pregnant mares' serum (Gonadogen, Upjohn) on the response of the seminal vesicles of underfed or vitamin-deficient male rats. It is presumed that the ovaries of young female rats would have responded similarly to the same experimental conditions.

The experiments reported were performed on weanling male rats of the Michigan State College and Carworth strains. In each experiment, uniform groups of rats were placed on special diets for a period of twenty-eight days. During the last four days of the experimental period, all except the control rats were injected with one half Cartland-Nelson unit (ten International Units) of pregnant mares' serum (P.M.S.). The animals were sacrificed on the twenty-eighth day, and body weights as well as weights of the seminal vesicles and coagulating glands were recorded. The testes weights were not recorded, since previous work (Meites and Chandrashakar, 1949) had indicated that P.M.S. had little effect on testes size.

A semi-synthetic diet was used in all except a few preliminary experiments in which it was desired to determine the effects of a commercial diet (Purina Laboratory Chow). The composition of the semi-synthetic diet was as follows:

Cerelose	62 gms.
Alcohol washed casein	25 "
Corn oil	5 "
Salt Mix. No. 2	4 "
Cod liver oil	5 "
Choline	100.0 mgm.
Ca Pantothenate	2.8 "
Niacin	1.0 "
Riboflavin	0.5 "
Thiamin	0.2 "
Pyridoxine	0.2 "
2,Me,1,4 Napthoquinone	0.04 "

Effects of reduced caloric or protein intake on seminal vesicle response to P.M.S. in Michigan State College rats

The results of this experiment are given in Table 4. The first four groups of rats were fed Purina Laboratory Chow. Group 2, which received P.M.S. showed approximately a 100 per cent increase (110.0±13.4 mg.)

in the weight of the seminal vesicles as compared to group 1, which received no P.M.S. (48.1±3.4 mg.). Groups 3 and 4 which received respectively only 3/4 and 1/2 of the feed consumed by the ad libitum fed rats of group 1, showed decreases in body weight and seminal vesicle response. However, on a 100 gm. body weight basis, the seminal vesicle response was the same as in the ad libitum fed rats.

The effects of the semi-synthetic diet on the reaction of the seminal vesicles to P.M.S. were similar to those obtained by feeding Purina Laboratory Chow. When caloric intake was reduced (groups 7 and 8), the body weights and seminal vesicle weights were correspondingly reduced, but the response of the latter to P.M.S. remained the same on a 100 gm. body weight basis. The reduction in protein (casein) intake also produced decreases in body growth and seminal vesicle response to P.M.S., but again the response of the latter to the gonadotrophin was the same on a 100 gm. body basis.

Effects of vitamin deficiencies on seminal vesicle response to P.M.S. in Michigan State College rats

These data are given in Table 5. Each of the vitamin deficiencies produced a decrease in growth, with the exception of group 9 which received no niacin. Inasmuch as this group, like the others in the experiment, received twenty-five per cent casein, it seems doubtful that a niacin deficiency was produced. Tryptophan which is present in large quantities in casein has been shown to be converted to niacin in the body.

The omission of vitamin A from the semi-synthetic diet evoked only a small decrease in the response of the seminal vesicles to P.M.S. (group 3). On a 100 gm. body weight basis, it appeared to be responsible for a significant increase in the response to P.M.S.

The omission of thiamin and pantothenic acid (groups 5 and 8) or riboflavin (group 6) from the diet, or the induction of folic acid deficiency by feeding one per cent of folic acid antagonist (group 10) or sulfasuccidine (group 12), all elicited significant decreases in the reaction of the seminal vesicles to P.M.S. The greatest inhibition in a seminal vesicle response to P.M.S. was produced in the riboflavin deficient rats. On a 100 gm. body weight basis, these rats appeared to show no response at all to P.M.S. The folic acid deficient rats showed the next greatest decrease in seminal vesicle response, while the effects of thiamin and pantothenic acid deficiencies were just on the borderline of significance.

Effects of folic acid antagonist and paired-feeding on a seminal vesicle response to P.M.S. in Carworth rats

The previous experiment demonstrated that folic acid and riboflavin deficiencies were particularly effective in inhibiting the response of seminal vesicles to P.M.S. This and the following two experiments were designed to determine whether deficiencies of these vitamins per se or the concomitant inanition evoked by their absence were responsible for the observed inhibition of seminal vesicle response to P.M.S. Inasmuch as Michigan State College rats were no longer available for these experiments, due to a failure in the animal room breeding program, Carworth rats were purchased and used.

TABLE 5

EFFECTS OF VITAMIN DEFICIENCIES ON SEMINAL VESICLE RESPONSE TO P.M.S. IN MICHIGAN STATE COLLEGE RATS

Group	No. of Rats	Diet Fed	Given P.M.S.	Average Initial Body Weight (gm.)	Average Final Body Weight (gm.)	Average Seminal Vesicle Weight (mg.)	Average Seminal Vesicle Wt. per 100 gm. Body Weight (mg.)
1	7	Semi-synthetic	No	43.0	156.0	85.5	54.8± 2.0 *
2	9	"	Yes	43.6	150.0	160.2	107.0± 10.5
3	8	No Vitamin A	"	43.5	83.9	135.7	157.2± 10.9 **
4	8	No Vitamin D	"	43.0	73.0	81.5	107.8± 18.2
5	6	No Thiamin	"	43.3	78.7	57.8	71.8± 13.1 **
6	4	No Riboflavin	"	43.3	54.0	26.6	42.8± 10.9 **
7	9	No Pyridoxine	"	42.6	91.9	85.2	93.0± 7.1 **
8	8	No Pantothenic Acid	"	43.9	90.7	72.7	80.3± 4.5 **
9	9	No Niacin	"	43.7	161.7	150.2	90.7± 8.0
10	9	Biotin Antag. 1 %	"	43.7	117.6	103.1	86.6± 6.9 **
11	10	Folic Acid Antag. 1 %	"	43.0	92.4	60.3	64.4± 7.2 **
12	8	Sulfasuccidine	"	43.9	100.0	65.6	65.6± 6.2 **

* Standard error of mean.

** Significant differences:

groups 2 and 3 - 2.09 groups 2 and 8 - 2.34

2 and 5 - 2.09 2 and 11 - 3.35

2 and 6 - 4.25 2 and 10 - 1.62

2 and 7 - 1.10 2 and 12 - 3.39

TABLE 6

EFFECTS OF FOLIC ACID ANTAGONIST AND PAIRED-FEEDING ON SEMINAL VESICLE RESPONSE TO P.M.S. IN CARWORTH RATS

Group	No. of Rats	Diet Fed	Given P.M.S.	Average Initial Body Weight (gm.)	Average Final Body Weight (gm.)	Average Seminal Vesicle Weight (mg.)	Average Seminal Vesicle Wt. per 100 gm. Body Weight (mg.)
1	8	Semi-synthetic	No	52.8	152.7	170.0	123.3± 12.3 *
2	7	"	Yes	52.8	153.4	309.8	202.3± 12.8
3	8	Folic Acid Antag. 0.5 %	"	52.1	101.6	121.0	117.7± 4.3
4	10	Pair-fed as above group	"	51.1	99.4	138.4	139.0± 9.7
5	8	Folic Acid Antag. 0.5 %	"	52.5	95.6	124.0	127.5± 9.5
6	9	Pair-fed as above group, plus 10-fold increase in B vitamins	"	52.5	99.4	148.5	149.8± 10.8

* Standard error of mean.
Significant differences : groups 3 and 4 — 2.00
groups 5 and 6 — 1.55

Folic acid deficiency was induced by feeding the antagonist. The same amount of feed consumed daily by the vitamin deficient rats was fed to controls, but without folic acid antagonist or sulfasuccidine. The results are shown in Table 6.

In order to insure that pair-fed rats received an adequate B vitamin intake despite reduced caloric intake, these vitamins were increased in the semi-synthetic diet ten-fold above the amounts ordinarily given and fed to group 6. Although the response in this group was slightly greater than in folic acid deficient rats (group 5), there was no statistically significant difference between the two groups.

Effects of riboflavin deficiency and paired-feeding on seminal vesicle response to P.M.S. in Carworth rats.

This experiment was performed in order to determine to what extent inanition was responsible for the reduced response to P.M.S. previously noted in riboflavin deficient rats. The results are given in Table 7. It can be seen that when riboflavin was omitted from the diet (group 3), there was a significant reduction in the body weight and in the response of the seminal vesicles to P.M.S. However, the pair-fed rats (group 4) showed a comparable response to P.M.S. on a 100 gm. body weight basis.

The effects of riboflavin deficiency and paired-feeding were further compared (groups 5 and 6), except that in the pair-fed rats the vitamins (B) were increased ten-fold to insure an adequate vitamin intake. The response of the seminal vesicles to P.M.S. in the groups of rats was the same on a 100 gm. body weight basis.

Ovarian hormones

One of the most interesting developments in the study of hormonal-nutritional interrelationships is the recent finding by Hertz (1945, 1946, 1948) that the response of the oviduct to estrogens is impaired when the diet is deficient in folic acid. This has been observed in the chick, rat, and monkey. Riboflavin, pyridoxine, and pantothenic acid deficiencies in the chick did not inhibit the response of the oviduct to estrogen, indicating the effects produced by a deficiency of folic acid were not simply due to reduced body growth and debility. These findings have been confirmed by Haque et al. (1949), Kline and Dorfman (1951), and others.

Velardo and Hisaw (1952, 1953) have also presented evidence that a deficiency of folic acid, elicited by feeding an antagonist of folic acid (aminopterin), can inhibit the action of progesterone on the uterus of the ovariectomized mouse, and can also prevent decidual development in intact and progesterone-treated, ovariectomized, pseudopregnant rats.

Astwood, Geschickter, and Rausch (1937) observed that the mammary glands of young rats on a restricted diet failed to respond to estrogen stimulation. Trentin and Turner (1941) similarly found a reduced mammary response to estrogen in underfed mice. It is possible that these results may have been due primarily to a decrease in availability of folic acid rather than to reduced caloric intake.

TABLE 7

EFFECTS OF RIBOFLAVIN DEFICIENCY AND PAIRED-FEEDING ON SEMINAL VESICLE RESPONSE TO P.M.S. IN CARWORTH RATS

Group	No. of Rats	Diet Fed	Given P.M.S.	Average Initial Body Weight (gm.)	Average Final Body Weight (gm.)	Average Seminal Vesicle Weight (mg.)	Average Seminal Vesicle Wt. per 100 gm. Body Weight (mg.)
1	8	Semi-synthetic	No	52.8	152.7	170.0	121.3± 12.3 *
2	7	"	Yes	52.8	153.4	309.8	202.3± 28.1
3	10	No Riboflavin	"	51.1	83.2	126.9	148.7± 15.3
4	10	Pair-fed as above group	"	52.2	99.0	146.2	147.2± 14.1
5	9	No Riboflavin	"	52.8	80.2	138.7	167.3± 14.4
6	9	Pair-fed as above group, plus 10-fold increase in B vitamins	"	52.8	91.2	152.8	166.3± 7.5

* Standard error of mean.

EFFECTS OF NUTRITION ON METABOLISM OF HORMONES

Hormone levels in the body are the result of a dynamic equilibrium between rates of production, inactivation, and excretion. The ability to inactivate hormones depends to a large degree on proper nutrition. If the diet is deficient in certain factors, this can lead to exaggerated effects on the reproductive system from either endogenous or administered hormones.

Zondek (1934) first showed that the liver could inactivate estrogens, and this has been widely confirmed. Other workers showed that progesterone (Kochakian, Haskins, and Bruce, 1944), androgens, and adrenal steroids (Burrill, and Green, 1942) can also be inactivated by the liver. Little is yet known of the metabolism of anterior pituitary hormones in the body. Biskind (1946) and Segaloff et al. (1944) found that on diets lacking in vitamin B-complex, thiamin, or riboflavin, the liver failed to inactivate natural estrogens. Drill (1946), however, demonstrated that the loss of power to inactivate estrogens during vitamin B deficiencies was due to the concomittant inanition rather than to lack of vitamins per se. He showed that animals given abundant vitamins but restricted in food intake were unable to inactivate estrogens.

Apparently an adequate protein intake is also necessary for liver inactivation of estrogens. Jailer and Seaman (1950) reported that rats fed a 50 per cent casein diet could still destroy estradiol, even when the diet was deficient in B vitamins, or during inanition. Under the same conditions, animals fed a 5 or 15 per cent casein diet could not inactivate estrogens. Vanderlinde and Westerfeld (1950) also noted that adequate protein is an important factor in maintaining the estrogen inactivating system in the liver.

Observations by Gyorgy (1945) indicate that lipotropic factors may also be essential to the liver for destruction of steroid hormones. He found that liver inactivation of estrogens was prevented on a high-fat low-protein diet, and that this could be overcome by adding methionine to the diet. One wonders what role vitamin B_{12} might play in the inactivation of steroid hormones, since it too is a lipotropic agent.

EFFECTS OF HORMONAL IMBALANCES ON NUTRITIONAL REQUIREMENTS

The endocrine glands are very important in the regulation of the metabolism of foodstuffs in the body. Thus the thyroid influences the absorption, utilization, and excretion of all nutrients; the adrenal cortex affects salt and water metabolism, protein synthesis, and gluconeogenesis; the pancreas is essential for normal glucose utilization; the parathyroids are important for Ca and P metabolism, etc. When physiological levels of hormones are present in the body, and all else remains equal, one may expect little or no change in normal dietary needs. But what will be the effect on nutritional requirements if there is a marked excess or deficiency of a particular hormone in the body accompanied, as likely as not, by profound alterations in secretion rates of other hormones? This question is of practical as well as of academic interest because of (a) the occurrence of spontaneous dysfunction of endocrine glands, and (b) the

increasing use of hormones, often in large dosages and over extended periods, in human and animal practice. If marked deviations in the endocrine balance can induce or aggravate dietary deficiencies, then supplementing diets with the necessary factors should help prevent or correct these deficiency symptoms (Meites, 1952a).

The thyroid has probably been more intensively investigated in relation to dietary needs than other endocrine glands. Thus, hyperthyroidism increases the need for most nutrients, including calories, minerals, and certain vitamins, while hypothyroidism is believed to reduce requirements for these factors. An example of the effects of hyperthyroidism on requirements for a specific factor, vitamin B_{12}, is shown in Fig. 1. Im-

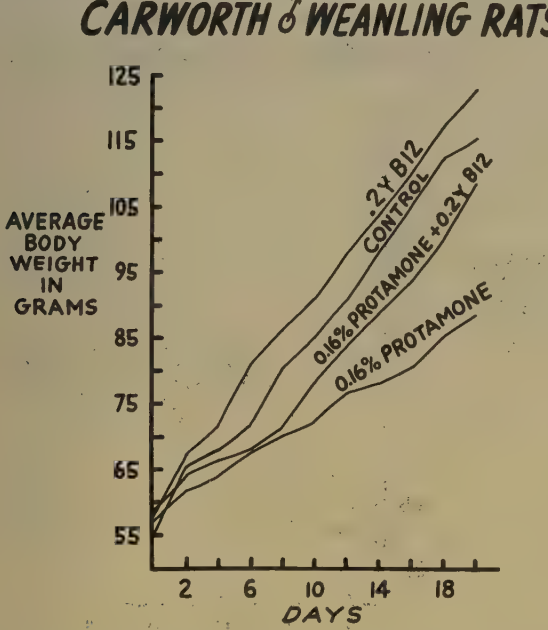


Fig. 1. Effects of feeding 0.16 % Protamone with or without injections of 0.2 micrograms of vitamin B_{12} daily on body growth of young rats fed an adequate diet.

mature albino male rats were divided into uniform groups by weight and were fed a basal ration which was adequate in all respects (contained 20 per cent whole milk powder). Hyperthyroidism was induced by incorporating 0.16 per cent Protamone[1] in the ration. It can be seen that Protamone reduced growth considerably and that this was partially counteracted

[1] An iodinated casein product containing thyroxine. Manufactured by Cerophyl Laboratories, Kansas City, Missouri.

by injecting 0.2 micrograms of vitamin B_{12} daily. Ershoff (1947) reported that the inhibitory effects of hyperthyroidism on the ovaries of young rats could be overcome by liver feeding. There are numerous data indicating that the thyroid helps regulate the conversion of carotene to vitamin A (Drill, 1943; Drill and Truant, 1947). The latter workers noted that thyroidectomized rats receiving only carotene developed xerophthalmia, whereas intact rats receiving only carotene remained normal. In view of the importance of vitamin A for the maintenance of pregnancy, the possibility must be considered that a hypothyroid state combined with a low intake of vitamin A could result in abortion or dead or weak offspring.

The effects of feeding 0.1 per cent diethylstilbestrol on requirements for vitamin B_{12} are shown on Fig. 2. These young male rats were fed the

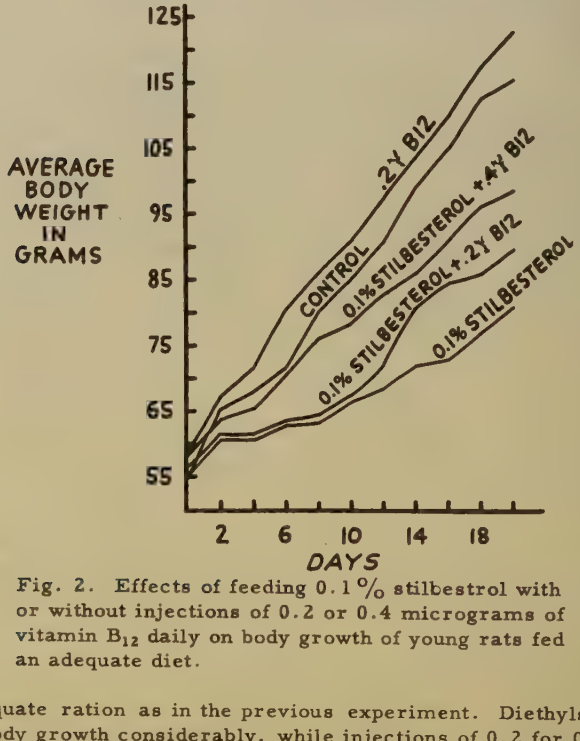


Fig. 2. Effects of feeding 0.1% stilbestrol with or without injections of 0.2 or 0.4 micrograms of vitamin B_{12} daily on body growth of young rats fed an adequate diet.

same adequate ration as in the previous experiment. Diethylstilbestrol reduced body growth considerably, while injections of 0.2 for 0.4 micrograms of vitamin B_{12} daily partially counteracted the growth inhibition. Ershoff (1947) observed that in immature rats fed massive doses of alpha estradiol, the inhibition of ovarian development could be counteracted by feeding yeast or desiccated whole liver.

Figure 3 shows the effects of combinations of Protamone and diethylstilbestrol on body growth, and their partial counteraction with vitamin

B_{12}. These experiments show therefore that hyperthyroidism or hyperestrinism can increase normal requirements for vitamin B_{12} in the young growing male rat. Injections of large doses of cortisone have also been shown to increase dietary needs for vitamin B_{12} in the young rat (Meites, 1951, 1952b).

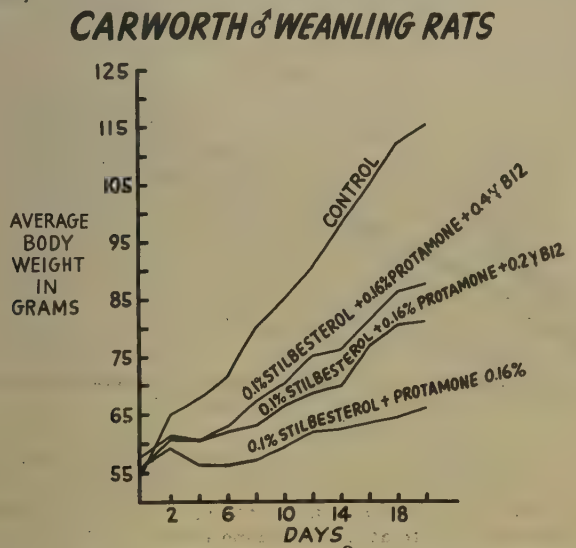


Fig. 3. Effects of feeding of 0.16 % Protamone and 0.1 % stilbestrol with or without 0.2 or 0.4 micrograms of vitamin B_{12} on body growth of young rats fed an adequate diet.

An example of the influence of a marked deficiency of two hormones in young rats on the need for vitamin B_{12} is shown in Figure 4. Young male rats were thyroparathyroidectomized and permitted two weeks for recovery. During this period they were fed an adequate diet and injected daily with calcium gluconate to prevent tetany. At the end of two weeks they were placed on a vitamin B_{12} deficient diet. Of 18 control rats subjected to parathyroidectomy, 15 survived for 30 days, 5 for 60 days, and all were dead before 90 days. Of 26 rats parathyroidectomized and fed either 50 or 200 micrograms of vitamin B_{12} per kilo of ration, 21 survived for 30 days, 19 for 60 days, 17 for 90 days and 16 for 120 days. The vitamin also enabled these rats to grow, although at a considerably slower rate than the intact rats given vitamin B_{12}. Further evidence that the inhibitory effects of hypothyroidism on growth can be partially counteracted by vitamin B_{12} comes from experiments in rats (Meites, 1950) and chicks (Libby and Meites, 1952) with thiouracil. These data suggest therefore, that insofar as survival and growth are concerned, thyroparathyroidectomy aggravates a deficiency of vitamin B_{12}.

The next experiment shows the effects of several nutrients on survival in adrenalectomized rats (Table 8). All of these rats, starting at 4 weeks of age, were fed a basal ration deficient in vitamin B_{12} for 30 days prior

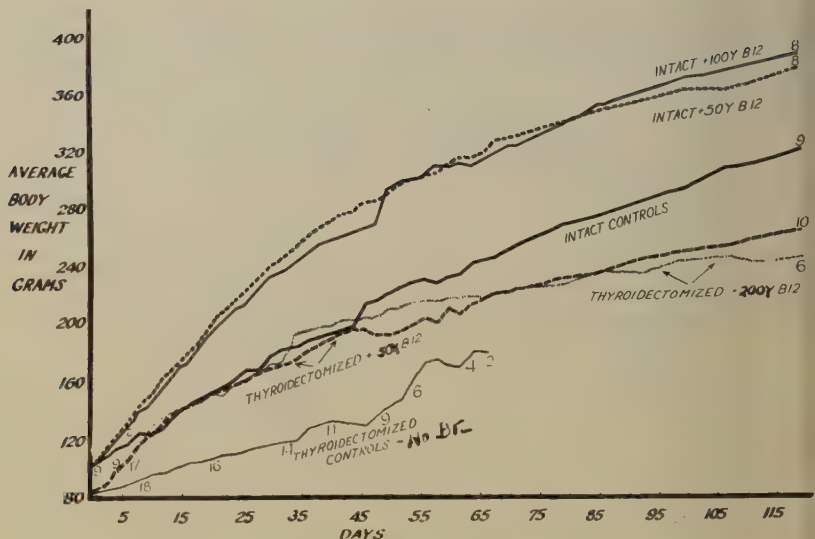


Fig. 4. Effects of thyroparathyroidectomy on growth and survival of rats fed diets deficient or adequate in vitamin B_{12}. The numerals underneath each growth curve indicate the number of rats at the beginning and end of the experiment.

to adrenalectomy, and 1 per cent NaCl was included in their drinking water after adrenalectomy. Half of the controls survived for an average of 7 days (median survival). Half of the group receiving vitamin B_{12} survived for 26 days, or 3.7 times as long (survival index). Vitamin B_{12} and aureomycin in combination increased survival time 9-fold above that of the controls, while a combination of vitamin B_{12} and lecithin proved slightly better in prolonging survival. The surviving rats usually showed some increase in body weight, although this did not compare with that of intact rats. Ralli and Dumm (1952) and Dumm and Ralli (1948, 1953) similarly observed that large doses of pantothenic acid, biotin, ascorbic acid, folic acid, and vitamin B_{12} can prolong survival time in adrenalectomized rats. These studies on parathyroidectomized and adrenalectomized animals suggest that dietary factors may substitute to some extent for hormonal deficiencies, and thus help maintain homeostatic mechanisms in the body.

SUMMARY AND CONCLUSIONS

Few nutritional factors have thus far been shown to conclusively influence reproductive functions. These include undernutrition or any condition producing loss of appetite, including deficiencies of B-vitamins, and lack of vitamins A, E, or folic acid. Factors which have not yet definitely

TABLE 8

INFLUENCE OF SEVERAL DIETARY FACTORS ON SURVIVAL RATE OF ADRENALECTOMIZED RATS

Treatment	Days on Experiment										Median Survival (Days)	Survival Index †
	0	10	20	30	40	50	60	70	80	90		
	No. of rats surviving											
Controls	15	4	0	-	-	-	-	-	-	-	7	1.0
Vit. B_{12} *	10	6	6	4	4	4	4	4	4	4	26	3.7
Vit. B_{12} + Aureo. ††	10	7	7	6	6	6	6	4	3	3	63	9.0
Vit. B_{12} + Lec. **	10	8	8	7	7	7	7	5	3	3	74	10.5

† "Survival Index" is the median survival of a treated group divided by the median survival of the untreated controls.
* 200 µg vit. B_{12} per kilo of diet.
†† .005 % Aureomycin (Lederle).
** 2 % Lecithin Compound, containing 50 % lecithin (Wyeth, Inc.).

been shown to be essential include unsaturated fatty acids and proteins. The demonstration that administration of B-vitamins to starved animals does not return sexual functions to normal does not prove that they have no role in reproduction. One B-vitamin, folic acid, has already been shown to be essential in several species for the growth response of the oviduct to estrogen and more recently to progesterone. The possibility cannot be excluded that other B-vitamins may be required for specific endocrine-reproductive functions. When one considers the basic role of the B-vitamins in the normal metabolism of protein, fat, and carbohydrate, it is difficult to believe that they are not at least as necessary for the growth and function of reproductive tissues as for other body tissues.

The only type of malnutrition which has conclusively been proven to impair the secretion of gonadotropic hormones by the pituitary is underfeeding or any state leading to inanition, including inadequacies of B-vitamins. Samuels (1948) and others believe that adequate dietary protein is also essential for normal anterior pituitary activity, although it is difficult to conceive that the minute amounts of protein needed for the synthesis of protein hormones by the pituitary cannot come from endogenous sources. Thyroid function is also decreased by undernutrition via the pituitary, but in addition it can be depressed directly by lack of iodine or by presence of goitrogenic substances in the diet. Plants of the family Brassicaceae appear to be outstanding in this latter respect, although many other foods have not yet been tested.

There is considerable basis for the belief that the gonads and sexual activities are more easily impaired during inanition than other body functions. In humans, it is well known that starvation may lead to reproductive disturbances (famine amenorrhea) before other body disorders appear. In rats, a loss of 15 per cent in body weight may stop the estrous rhythm. The fact that the ovaries remain responsive to stimulation by gonadotropic hormones during underfeeding does not necessarily imply that normal estrous cycles and pregnancy could be maintained merely by administering the essential hormones. Some of the effects of undernutrition are probably exerted directly on the reproductive tissues, although the major action is probably through the endocrine glands. The effects of deficiencies of vitamins A or E, however, appear to be direct on the reproductive tissues *in utero* during pregnancy, and the effects on the endocrine glands, when observed, are probably secondary in nature.

When liver function is impaired by nutritional or other means, inactivation and conjugation of steroid hormones may be greatly decreased. Since the production of gonadal hormones is also decreased during inanition, liver function may not be of much importance to reproduction during reduced food intake. On the other hand, this could be of considerable importance when steroid or gonadotropic hormones are administered to underfed or starved animals.

Hormonal imbalances may impair reproductive activities by several means. Thus hypo- or hyperthyroidism may reduce the secretion of gonadotropic hormones by the pituitary, alter gonadal response to gonadotrophins, and change dietary requirements. Hypothyroidism may prevent the conversion of carotene to vitamin A. Dysfunction of other endocrines may also alter nutritional needs. It is unfortunate that sensitive, reliable assay methods have not yet been developed for measuring hormone levels

in the body. However, these studies of the effects of hormonal imbalances on nutritional requirements emphasize the desirability of feeding an adequate diet for health and reproductive purposes.

Most of the advances in the fields of nutrition, endocrinology, and reproductive physiology have been made since the first World War. This fact, plus the tendency towards specialization, has resulted in a dearth of nutritionists with a first hand knowledge of endocrinology. In view of the importance of both fields to the study of reproductive and other functions of the body, it is hoped that the future will see an increase in the number of nutritionist-endocrinologists, as well as closer cooperation between the two groups of workers. More research can then be directed towards defining the specific points at which nutrition, hormones, and reproductive tissues interact.

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GONADOTROPHIC ACTIVITY OF PITUITARY GLANDS AND THE INDUCTION OF OVULATION

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A quarter of a century ago the dependence of the gonads on the pituitary gland for hormonal stimulation and support was demonstrated. Following this pioneering work, many studies on the gonadotrophic potency of anterior pituitaries were reported, and attempts were made to relate pituitary potency to species, age, sex, and reproductive state. It is not the purpose of this discussion to bring together all the information available on this subject. Attention will be centered on those aspects of the problem which should be of particular interest to this audience, and which are largely an outgrowth of studies conducted in our laboratory on domestic animals. For reasons which shall be mentioned later, such studies seem more informative and easier to interpret than some of the work done on laboratory mammals with short estrous cycles.

Soon after the inception of the bioassay method for determining the gonadotrophic potency of pituitaries, the following questions were asked by endocrinologists: What are we measuring when we are assaying pituitary glands? Are we measuring the actual rate of secretion of the hormones? Are we measuring the amounts of hormones which happen to be stored in the gland at the time when it was obtained for assay, and which have not yet been released? Or are we determining the residual amount of hormone following release of the major part of it?

The answers to these questions are important because the quantity of either stored hormone or residual hormone appears to bear little relation to the rate of gland function at any given reproductive stage. Only if there is found to be a close correlation between the potency of the pituitary gland and the reproductive state, are we justified in assuming that the amount of hormone found in the assayed glands bears any relationship to normal or abnormal rates of gonadal function.

As far as the gonadotrophic hormones are concerned, the pituitary gland may function in one of several ways, two of which are sufficiently plausible to merit discussion. At any given time the gland could be producing and storing hormones regardless of the needs of the target tissues. To permit cyclic behavior, the release of such stored hormones could take place in response to humoral or neural stimuli coming from the gonads, or from the rest of the reproductive system. In the second instance, the rate of hormone secretion could be regulated strictly by the needs of the target tissue. This assumption would rule out the possibility of storage of hormones for possible future use. The release mechanism again could

be neural and/or humoral. Thus it seems important to begin this discussion by proposing an answer to the questions raised. This can best be done by comparing pituitary gland potencies with the reproductive performance within individuals. To permit statistically valid comparisons, sufficiently large numbers of animals, which are known to have had normal reproductive cycles, should be sampled throughout the cycle at close intervals.

To fulfill these conditions, sows and gilts were killed on almost every day of the cycle, and the relationship between the gonadotrophic hormone content of individual pituitary glands and the ovarian activity of the individuals from which these glands came were determined (Robinson and Nalbandov, 1951). The pig seems especially suited for such a study because of the fact that it is a polytocous animal, has a long estrous cycle with distinct luteal and follicular phases which are easily distinguishable and are not telescoped together as they are in some laboratory animals. It also has a sufficiently large ovary permitting accurate morphological measurements. Most important, it has a large pituitary gland which permits replicated assays of individual glands on several assay animals. Because of the large numbers of pituitaries assayed in this study, and because of the uniformity of the data obtained, the results which showed statistical significance deserve detailed analysis and comment.

The most important finding, from the point of view of the question posed earlier, is the very close agreement between the gonadotrophic potency of the assayed glands and the follicular activity of the corresponding ovaries. This correlation was found to be +0.69 and highly significant at less than 1.$^{o}/_{o}$ (Table 1). Results similar to these were obtained on sheep during the estrous cycle.

Approaching this problem from a different point of view and using different methods, Byrnes and Meyer (1951) concluded that in adolescent rats ". . . there was a very close correlation between the gonadotrophic hormone content of the pituitary and the hormone which had been secreted into the blood stream."

On the basis of these and similar findings, the answer to the question posed earlier is that the assay of the pituitary gland does indeed measure the rate at which the gonadotrophic complex is being produced and secreted into the blood stream at the time of autopsy of the animal. It is very probable that there is a temporal lag between the elaboration of the hormone and its effect on the ovary (as has actually been shown for the release of the ovulating hormone, LH, and ovulation), but there is good reason to assume that this time lag amounts to hours rather than days. Thus, it seems probable that the gland makes and releases gonadotrophic hormones in response to the needs and the dictates of the target organ, and it seems unlikely that there is any storage of gonadotrophic hormone in the pituitary glands of normal cycling females.

Unfortunately, no good and reliable bioassay methods are available for the in vivo separation of FSH and LH effects of individual pituitary glands. Most of the discussion to follow will pertain to the gonadotrophic complex. What conclusions can be drawn with regard to the quantitative relationships between FSH and LH during the various reproductive states will have to be based on circumstantial evidence.

TABLE 1

Changes in the Proportion of Follicles of Different Sizes During the Estrual Cycles of 33 Females.

Day of Cycle	Per cent Follicles in Class (Follicle size (mm))					Av. No. Follicles in Both Ovaries	Gonadotrophic Hormone in Pituitary[1]	Cycle
	5	5-7	8-10	10	Cysts			
4	100.0					13	14.7	Luteal
5		100.0				19	13.5	phase
6	56.2	39.7			4.1	24	17.2	
7	47.4	52.6				19	14.1	
8	74.5	25.5				49	23.5	
9		88.8			11.1	49	29.6	
10	61.5	38.5				42	26.1	
13	67.9	30.1			2.0	51	28.1	
14	51.4	46.0	2.7			37	22.5	
16	39.5	59.3	1.2			40	23.1	Follic-
17	47.2	51.7	1.1			45	26.4	ular
18	56.4	33.3	10.3			39	33.5	phase
19	43.9	8.7	47.4			57	20.5	
20	83.9	16.1				62	22.5	
1	5.1	8.9	76.0	8.9	1.3	16	13.7	Heat
1	-	-	69.2	30.8	-	13	17.1	Heat

[1] Testes weight, mgs.

Rates of gonadotrophic hormone secretion during the estrous cycle.

A number of studies has been made on this subject and, in general, there is good agreement with regard to the conclusions drawn. The most recent work was done on sheep (Kammlade et al. 1952), and on pigs (Robinson and Nalbandov, 1951). These studies have the advantage over older information in that the pituitary gland samples were obtained at frequent intervals during the estrual cycle, so that almost every day of the cycle was represented in the assay. Furthermore, the glands in both studies were assayed individually, making it possible to correlate the gland potencies with the ovarian activity of the same individual.

Swine. The data obtained on swine bring out a number of interesting points which can be summarized as follows (Table 1):

1. The close correlation between the gonadotrophic potency and the ovarian activity (r=+0.69) has already been pointed out and its significance discussed.

2. Animals in heat have pituitary glands with a very low gonadotrophic content (see also Paredis, 1950, for the cow). This finding is compatible with the theory that FSH secretion has been inhibited by increasing amounts of follicular estrogen. It is assumed that in the animal in heat the pituitary gland produces primarily LH, which would be expected to cause less of a

quantitative response in the end-organs of assay animals, than would FSH.

3. During early luteal phase (days 3 through 7) of the cycle, the gonadotrophic potency remains low. It is difficult to explain this low titer by invoking estrogen inhibition of the pituitary, primarily because the estrogen level during that period is also low. It could be assumed that the pituitary has not yet recovered from the inhibition during the preceding follicular phase, but the gland recovers under certain experimental conditions from much larger dosages of estrogen in a shorter time. It also seems significant that both the gonadotrophic potency and the ovarian activity increase not gradually but abruptly on about the eighth day of the cycle.

After the sudden rise on the eighth day of the cycle, the gonadotrophic potency remains consistently and uniformly high until day 20, when it drops just as abruptly and to the same amount as it rose on day 8. This drop coincides with the onset of estrus.

Because of the abruptness of the changes of both the ovarian morphology and the gonadotrophic potency, and because of the very close correlation between these changes, the question arises whether some control mechanism other than estrogen may not be involved in controlling the gonadotrophic hormone output by the pituitary gland. It is possible to postulate control mechanisms other than estrogen, and adduce experimental evidence for their support, but additional work will be needed before revision of the classical estrogen theory of pituitary control can be adequately documented.

4. It was surprising to learn that the number of follicles is very closely correlated with pituitary potency while the size of the follicles is not (Table 1). As late as day 18 of the cycle, over 90 % of the follicles are smaller than ovulatory size. It appears possible that the ovulatory spurt does not take place until the first day of heat, when, concomitant with the increase in follicular size, there is a drastic decrease in follicle number to that number of follicles which are destined to ovulate. It is possible to interpret these ovarian changes by assuming that the initial increase in follicular size is due to a gradually increasing output of LH. The sudden drop in follicle number shortly before heat may be caused by unchanged rates of FSH production rather than FSH inhibition by estrogen. Thus, as follicles increase in size, not enough hormone is available to maintain all of them and most of them become atretic. This invokes the principle of "hormone dilution" which has been advanced as the most likely explanation of growth stasis at physical maturity of animals (Baird, Nalbandov and Norton, 1952), and which may apply in this instance. In any event, while this interpretation fits the observed facts, no actual assays for the changes which occur at that time in the levels of FSH and LH are available.

In neither pregnant nor cycling pigs was there any correlation between the gonadotrophic potency of the pituitary and the number of eggs ovulated (corpora lutea), or the litter size. This is in sharp contrast to the highly significant correlation of +0.69 between the number of ripening follicles, or the number of follicles destined to ovulate, and the pituitary gonadotrophic potency. The disassociation between efficiency of ovulation and gonadotrophic potency is open to several interpretations. Ovulation may be brought about as effectively by large amounts of LH as it is by traces

of that hormone; or the amount of LH to be released may be the same regardless of the number of follicles to be ovulated, which accords well with the idea that LH release may be a neuro-humoral phenomenon even in spontaneously ovulating animals; or, finally, the assay methods employed may be too crude to measure minor changes in LH potency of pituitary glands.

Sheep. A study, similar to the one described for swine, was done on sheep (Kammlade et al. 1952). In this case a comparison was made between ovarian morphology and the changes in the gonadotrophic potency of pituitaries on almost every day of the estrous cycle, and the gonadotrophic potency during the non-breeding season. The following conclusions were drawn:

1. During the estrous cycle the results obtained for sheep are very similar to those discussed for swine, with one important exception. Whereas in swine a close correlation was found to exist between gonadotrophic potency and follicle number, in sheep a significant correlation existed between gonadotrophic potency and follicle size but not follicle number. It should also be noted that in swine both follicle size and follicle number change rather abruptly during the cycle, while in sheep this change is linear and gradual throughout the cycle. These differences may be a reflection of the fact that sheep are monotocous, while pigs are polytocous animals.

2. Of particular interest was the finding that, contrary to the accepted assumption (Robinson, T. J., 1950), sheep pituitaries contain significantly more gonadotrophic hormone during the anestrous season than they do during the estrous cycle. During the nonbreeding season the ovaries are far from being inactive. The average diameter of the follicles and the diameter of the largest follicles were the same during the two reproductive phases. There was, however, a significant difference in the total number of follicles found (18.96 ± 7.01 during anestrus and 26.18 ± 13.01 during the breeding season).

On the basis of these findings it was postulated that the anestrous period in sheep is not due to a deficiency of the gonadotrophic complex, but instead may be due to an imbalance between FSH and LH. An educated guess would be that the anestrous period is caused by a rise in FSH and a fall in LH. This guess is in part supported by the facts that the ovaries are not inactive during the anestrous period and that some ewes can be made to ovulate with LH during that period. Because of the findings of Yeates (1949) and others, that the onset of the breeding season seems to be closely tied to decreasing daylight, while anestrus follows increasing amounts of light; it appears plausible that the increase in FSH is brought about by the increasing stimulation of the pituitary gland by light. The balance between FSH and LH is restored by the reduction of the rate of FSH secretion in response to diminishing hypophyseal stimulation by light.

Rates of Gonadotrophic Hormone Secretion During Pregnancy.

Determination of the gonadotrophic potency of pituitaries during pregnancy throws additional light on the pituitary-gonadal interrelation. One such study has been conducted on cows (Nalbandov and Casida, 1940), while another concerns itself with swine (Robinson, G. E., 1950). The

results obtained in these two species are strikingly similar. A steady and significant decrease in gonadotrophic activity of glands occurs from early to late pregnancy. This decrease is probably due to the well known inhibiting action of estrogen. In this instance the decreasing gonadotrophic potency is inversely related to the increasing amounts of placental estrogen. The progressive decline in follicular development observed in both species suggests that estrogen inhibits the production rather than the release of the gonadotrophic hormone.

In both studies an inexplicable relationship was found to exist between the sex of the fetus and the potency of the glands of the mother. Cows carrying female fetuses, and sows whose litters were predominantly female, had pituitaries which were more potent throughout pregnancy than the glands from females carrying male fetuses or whose litters consisted predominantly of males. There is no obvious explanation for this phenomenon which seems to be a real one and which operates in a monotocous as well as in a polytocous species.

INDUCTION OF OVULATION

The traditional concept of pituitary-ovary relationship is too well known to need review here. The fact that LH, acting on the mature follicle, is the immediate cause of ovulation has been amply demonstrated in many species. However, the trigger mechanism responsible for the release of the ovulating hormone is still under discussion. Experimentally LH release has been accomplished by estrogen and progesterone, and it has been well established that a neurohumoral release of this hormone occurs under normal conditions in a number of species--in birds as well as in ovulating mammals.

The backbone of the traditional theory of ovary-pituitary relationship is the control exerted over the pituitary gland by estrogen. The recent study of Byrnes and Meyer (1951) corroborated the observation made long ago that estrogen inhibits the production or the release of FSH. This work also puts this effect within the limits of physiological plausibility by showing that minute quantities of estrogen are capable of accomplishing this inhibition. There is also evidence that estrogen is able to induce ovulation presumably through release of LH. Ovulation follows maximal follicular growth and estrogen secretion. This theory seems attractive as the LH releasing hormone operating in the normal cycling female.

As a rule, ovulation does not occur during pregnancy, although it can be induced experimentally and is known to have happened spontaneously. Because considerable follicular development is noted in most pregnant females, and because in both the cow (Paredis, 1950), and the sow (Robinson and Nalbandov, 1951) the pituitaries of pregnant females were found to be as potent as those from non-pregnant females during the follicular phase of the cycle, therefore, failure of ovulation during pregnancy has been ascribed to the LH inhibiting action of progesterone. That it does so in non-pregnant females has been shown for several laboratory animals (see Everett, 1948) and more recently for domestic animals. Daily dosages of progesterone varying from 10 to 50 mgs. will prevent ovulation in sheep (Dutt and Casida, 1948), cattle (Ulberg, Christian, and Casida, 1951), and in pigs (Ulberg, Grummer, and Casida, 1951).

These data are in direct contradiction to equally impressive results which show that progesterone, under certain conditions, is capable of causing ovulation rather than preventing it. This has been demonstrated in rats and rabbits (see Sawyer, 1952), laying hens (Fraps and Dury, 1943), and in the cow (Hansel and Trimberger, 1952). In view of these contradictory results, the temptation is strong to discount the LH inhibiting action of progesterone as being unphysiological in nature. The dosage of progesterone as well as the time of its administration appears to have an important influence on its ultimate effect as an LH releasing or inhibiting hormone.

It is tempting to speculate on the possible role of progesterone as an LH releasing hormone during the normal estrous cycle, especially in view of the fact that recent work has shown that progesterone appears in demonstrable amounts prior to ovulation in rabbits (Forbes, 1953) and guinea pigs.

An appreciable amount of work has been done in this laboratory on the neural control of the hypophysis in two spontaneously ovulating species, the sheep and the chicken. While the analysis of the situation in the ewe is not yet complete, two facts have been established to date. The cycle in the ewe can be significantly modified by the presence of a distending foreign body in the uterine horn. If a plastic bead is implanted three days after heat, the cycles are very significantly shortened causing frequent heats accompanied by ovulations (Moore and Nalbandov, 1953). If, however, a bead is implanted on the eighth day of the cycle, then the intervals between heats are appreciably lengthened and the corpus luteum is caused to persist beyond its normal life span. In both instances, denervation of the uterine region containing the bead causes the return of normal cycle length and ovulation. This suggests that we are dealing with a neurogenous mechanism operating via the hypothalamus. While the endocrine modifications produced in the pituitary by distention of the uterus remain unexplained, it is significant that the uterus and its contents can play a role in the modification of the rates of hypophyseal function, more specifically the rates of ovulation and follicle maturation and the maintainance of the corpus luteum. To what extent the uterus participates in these functions in the normal cycling or the pregnant animal remains to be determined.

A much clearer situation from the endocrine point of view exists in the laying hen, where the presence of an irritant in the oviduct specifically prevents the release of ovulating amounts of LH, without apparently interfering with the flow of the other hormones (Huston and Nalbandov, 1953). This is shown by the fact that an oviducal irritant stops ovulations for about 20 days without causing follicular atresia, changes in oviduct size, or change in comb size. These experiments were interpreted to show that the presence of an irritant (an egg?) in the oviduct, does not alter the rate of FSH secretion as evidenced by normal ovarian size and absence of follicular atresia. Neither does it reduce the steady flow of some LH (as shown by the normal comb size), but it does specifically inhibit the secretion of quantities of LH sufficient to cause ovulation which can be readily induced with exogenous LH at any time during the period when normal ovulations are prevented by the presence of the irritant. The possible role of this mechanism in the normal economy of the hen continues to be a subject for research, but it is cited here as an additional instance of the

control of the ovulating mechanism of the pituitary gland by the oviduct or its contents in a presumed spontaneous ovulator.

SUMMARY

Because of the close correlation which has been found between the gonadotrophic potency of pituitary glands and the follicular development of the ovaries throughout the estrous cycle, the conclusion is drawn that bioassay of pituitary glands accurately reflects the rate of hormone production and release.

The changes occurring in pituitary potency and ovarian morphology during the estrous cycle are abrupt. There is some question whether the estrous cycle of mammalian females is primarily regulated by the ebb and flow of follicular estrogen.

In neither pregnant nor cycling females was there any correlation between gonadotrophic potency and the number of follicles ovulated or the number of corpora lutea found.

The fact that the pituitaries of sheep during the non-breeding season are more potent than they are during the estrous cycle indicates that the non-breeding season is due to hormonal imbalance rather than hypophyseal inactivity. This is supported by observations on ovarian morphology during the two reproductive phases.

It is suggested that in the normally cycling, spontaneously ovulating females, release of the ovulating hormone is caused by progesterone which acts through a neurogenous mechanism.

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THE ROLE OF ESTROGENS AND PROGESTERONE IN OVULATION[1]

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The ovarian cycle consists essentially of the maturation of the follicle immediately before and during estrus, the rupture of the mature follicle at or near the end of estrus, the development of the ruptured follicle into a corpus luteum, and the subsequent atrophy of the corpus luteum during the interestrual period. In some forms the interestrual period is marked by obvious growth and regression of follicles with the larger follicles which are present at estrus being the ones which are usually singled out for preovulatory growth and rupture.

The factors directly responsible for at least the later stages of follicular development have been shown conclusively to be present in the pituitary gland. Smith and Engle (1927) demonstrated by transplanting pituitary glands into immature mice that premature maturation of the follicle along with precocious opening of the vagina and enlargement of the uterus could be induced. Factors responsible for the production or release of gonadotrophic hormones by the pituitary gland have been a matter of much interest and speculation for research workers.

The problem of pituitary and ovarian interrelationship has been investigated extensively during the past few decades and there seems to be general agreement that large quantities of gonadal hormones will suppress gonadotrophic hormone secretion. However, there is considerable controversy concerning the quantitative aspect of the effect of gonadal hormones on the secretion of gonadotrophic hormones. A most important consideration is whether or not rupture of the follicle is caused by the action of estrogen alone on the pituitary gland through release of luteinizing hormone, or whether progesterone is secreted by the granulosa cells of the follicle prior to ovulation and indirectly plays some part in release of the luteinizing hormone from the pituitary gland.

Any consideration of the effect of estrogen and progesterone on ovulation must include some knowledge of ovarian-pituitary relationship. Because of the lack of critical information in farm animals in this respect, it is necessary to review some of the experimental work with laboratory animals.

Effect of Estrogens on Ovulation

The work of Hohlweg (1934) indicates that estrogen acts on the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

pituitary gland, resulting in the release of luteinizing hormone, since the ovaries of immature female rats autopsied after a series of estrogen injections contained numerous corpora lutea. Hohlweg also found that in mature rats repeated injections of estrogen resulted in enlargement of the anterior lobe of the pituitary gland, which, after being implanted into immature females, resulted in luteinization of their ovaries in contrast to only follicular development from untreated pituitary implants. Lane (1935) also has shown that the initial effect of estrogen on the pituitary gland is to stimulate the release of gonadotrophic hormone. This is followed, if estrogen injections are continued, by a decreased gonadotrophic output as measured by ovarian response to implanted glands. Byrnes and Meyer (1951) found that in rats follicle-stimulating hormone secretion was greatest when estrogen was at a very low level and that more luteinizing hormone was secreted when estrogen was present in larger amounts. In this study alpha-estradiol was injected within "physiological limits."

Fevold, Hisaw, and Greep (1936) reported similar observations in the rat and showed more definitely that the action of estrogen on the pituitary causes increased secretion of luteinizing hormone rather than follicle-stimulating hormone. They found that in immature rats estrogen augments the effect of a given dose of follicle-stimulating hormone, but estrogen did not augment the action of a luteinizing preparation, which would indicate that estrogen did not cause an increased secretion of follicle-stimulating hormone from the rat's own pituitary gland. No augmentation was observed by these workers in the hypophysectomized animal. They were unable to demonstrate any augmentation effect if estrogen was given for a period of eight days prior to the administration of follicle-stimulating hormone, and they concluded that the luteinizing hormone of the pituitary gland had been depleted by that time. Fevold and Fiske (1939) were able to demonstrate the formation of luteal tissue in the ovaries of adult rats by estrogen injections. They found that pituitary implants from estrogen-injected rats into infantile mice resulted in more estensive luteinization of the ovaries in the mice than did untreated pituitary implants.

There is not complete accord concerning the effect that estrogens have on the secretion of luteinizing hormone. Estrogens do not stimulate the release of luteinizing hormone in animals which do not ovulate spontaneously. Release of the luteinizing hormone in such animals is apparently under nervous control, and, at least in the rabbit, ovulation cannot be induced experimentally by estrogens (Bachman, 1936). A decrease in gonadotrophic hormone secretion by estrogen administration in the rat was reported by Moore and Price (1930), Meyer, Leonard, Hisaw, and Martin (1930), and others. Because of abnormal development of corpora lutea, estrogens will cause enlargement of the ovary, if it already contains corpora lutea, when compared with untreated control animals (Hohlweg, 1934). This enlargement of the ovaries has caused some confusion in which ovarian weight alone was used as the criterion of the effect. It is important that the formation of new corpora lutea should not be confused with maintenance of the old ones.

Based on experimental data, the cyclic nature of the reproductive activity in the rat has been accounted for on the basis of slight changes in the estrogen level in the blood, which reciprocally affect the secretion of follicle-stimulating and luteinizing hormones. It is postulated that, when

levels of estrogen are low, the pituitary secretes larger amounts of follicle-stimulating hormone. As the estrogen in the blood reaches moderate levels, follicle-stimulating hormone secretion is decreased and the pituitary gland is stimulated to secrete larger amounts of luteinizing hormone. Preovulatory follicular swelling and ovulation are thought to be due to an augmentation action of follicle-stimulating and luteinizing hormone. The above hypothesis is that proposed by Hisaw, Fevold, Foster, and Hellbaum (1934) and Fevold, Hisaw, and Greep (1936).

A most obvious effect of estrogens on the ovary, if given in doses large enough and administered over a period of time, is to cause inactivity and atrophy. Noble (1938) implanted crystals of stilbestrol into rats, and the atrophy of the ovaries resembled that which follows hypophysectomy. Assay of the pituitary glands of these animals showed them to be deficient in gonadotrophic hormone. A similar action of estrogen on the ovary has been recorded by Folley and Malpress (1944) in heifers, in which the ovaries became inactive and the gonadotrophic function of the pituitary gland was decreased. Implantation of stilbestrol or hexoestrol in cows and heifers checked ripening of the ovarian follicles in experiments carried out by Hammond and Day (1944). These results have been explained by the fact that large amounts of estrogen decreased the secretion of follicle-stimulating hormone. As a result, no mature follicles are present, since they depend upon this hormone for their existence and consequently ovulation will not occur.

It is apparent that dosage level of estrogens, duration of administration, and time of administration with respect to the stage of the estrual cycle are important. Large amounts of estrogen given over a period of time will result in inactivity of the ovaries. In laboratory animals, dosages within "physiological limits" appear to cause the gland to release luteinizing hormone resulting in ovulation. However, if the follicles are not previously matured sufficiently by the action of follicle-stimulating hormone, ovulation does not occur.

In recent years there has been considerable interest in the possibility of stimulating estrus and ovulation in anestrual ewes. Since the injection of estrogen has been found under certain conditions to stimulate ovarian activity in laboratory animals, it is possible that similar response may be obtained in larger animals. Hammond, Hammond, and Parkes (1942) were able to induce estrus and ovulation in three anestrual ewes injected with estradiol benzoate. Of twelve ewes injected with stilbestrol dipropionate, only three came into estrus and ovulated. However, three additional ewes ovulated. Hammond, J., Jr. (1945) reported that three of eleven ewes injected with stilbestrol ovulated, and it was thought the ovulation was induced only in ewes which had fair-sized follicles in the ovaries. Cole, Hart, and Miller (1945) found that ovarian activity was not stimulated in mature anestrual ewes following injection of estradiol benzoate. Vander Noot, Reece, and Skelley (1949) failed to induce lambing in anestrual ewes treated with estradiol propionate, even though the treatment was effective in inducing mating. The above results show that estrogens do not consistently produce ovulation when administered to anestrual ewes.

Only a few studies have been reported in which estrogens have been administered to larger animals at the onset of estrus and in which the

effect on the time of ovulation had been studied. If estrogens are responsible for release of the luteinizing hormone by the pituitary gland, then, injecting estrogens early in the heat period should hasten ovulation in farm animals. Hansel, Trimberger, and Bearden (1952) administered from 1000 to 3000 I. U. of estradiol at the beginning of heat in 14 dairy heifers. The average interval from onset of estrus to time of ovulation was 32.4 hours for the treated periods and 29.8 hours for control periods on the same animals. Simpson (1952) injected 1 mg. of estradiol benzoate into ewes at the onset of natural heat. The average interval from onset of estrus to time of ovulation in six treated ewes was 44 hours, compared to an average interval of 36 hours for a group of six control ewes. Time of ovulation was determined in this study by mid-ventral laparotomy at 24-hour intervals.

TABLE 1

Effect of Estradiol on Time of Ovulation

Type of Animal	Interval Onset of Estrus to Ovulation: Control Period (Hrs.)	Interval Onset of Estrus to Ovulation: Estradiol Treatment Period (Hrs.)	Source
Dairy Cattle (14 heifers)	29.8	32.4	Hansel, Trimberger and Bearden, J. Animal Sci. 11:793-794. 1952.
Ewes (6 per group)	36±2.5	44±4.7	Simpson, E. C., Master's Thesis, U. of Kentucky, 1952.

Failure of estrogen to hasten ovulation in these experiments may indicate that either a rising blood level of estrogen at time of estrus does not cause inhibition of follicle-stimulating hormone secretion and release of luteinizing hormone by the pituitary gland, or else, the dosage of hormone employed in these studies was above "physiological limits" for these animals. It does seem reasonable to assume that the follicles at this stage of the cycle were sufficiently developed to react to an ovulating stimulus.

These varied results in laboratory and farm animals indicate that, under certain conditions estrogens apparently increase, and under other conditions decrease the gonadotrophic activity of the pituitary gland. The exact relationship still remains obscure.

Ovulation Inhibiting Action of Progesterone

The theory of the inhibition of estrus by the corpus luteum was first tested experimentally by Loeb (1911), who demonstrated in the guinea pig

that the removal of the luteal tissue accelerated the next ovulation, whereas extirpation of other parts of the ovaries had no such effect. Williams and Williams (1921) and Hammond (1927) reported that extirpation of the functional luteal tissue from the cow resulted in estrus and ovulation occurring within two to four days. McKenzie and Terrill (1937) reported similar observations in the ewe.

Papanicolaou (1926) showed that a lipoid extract of luteal tissue inhibited estrus and ovulation in the guinea pig. Gley (1928) was able to inhibit estrus in the rat by extracts of sow corpora lutea and Parkes and Bellerby (1928) were able to inhibit estrus in the mouse by injecting extracts from corpora lutea from the cow. Allen (1932) reported the isolation of a potent crystalline preparation from luteal tissue and, a few years later, the isolation of pure crystalline progesterone was announced by several workers. The availability of a standardized preparation aided greatly in understanding the physiological action of the corpus luteum.

Makepeace, Weinstein, and Friedman (1936) found that 1 mg. of crystalline progesterone injected into non-suckled post-partum rabbits inhibited ovulation, and they concluded that progesterone was interfering with the release of the luteinizing hormone from the pituitary gland. They found that ovulation could be produced in the treated rabbits by injection of extracts from pregnancy urine. Similar results have been reported for the guinea pig (Dempsey, 1937) and the rat (Selye, Browne, and Collip, 1936, and Phillips, 1937). Astwood and Fevold (1939) have shown that pseudopregnancy in rats, which follows electrical stimulation of the cervical region, did not occur if progesterone was injected prior to the application of the stimulus. Thus, progesterone must have either blocked out the stimulus itself, or prevented the release of the luteinizing hormone from the pituitary gland.

More direct evidence that progesterone curtails the supply of gonadotrophic hormone from the pituitary gland is furnished by Burrows (1939). He injected progesterone into adult rats and then implanted their pituitary glands into infantile mice and found that their ovaries and uteri were smaller than those from control mice which had been implanted with pituitary glands from untreated rats. Biddulph, Meyer, and Gumbreck (1940) showed the capacity of progesterone to check the output of pituitary gonadotrophin in another manner. They placed in parabiosis two female rats, one of which had been ovariectomized. The increased output of pituitary gonadotrophin by the ovariectomized rat caused constant estrus in the intact partner. When 1 mg. of progesterone was given daily to the spayed partner, the hypersecretion of its pituitary was suppressed and the persistent estrus in the intact partner ended. This effect of progesterone in blocking ovulation is believed to act directly on the pituitary gland in inhibiting the production or release of luteinizing hormone.

In applying this knowledge to studies with larger animals, Dutt and Casida (1948) and O'Mary, Pope, and Casida (1950) were able to synchronize the estrual periods in ewes during their natural breeding season. By daily injections of 10 mg. of progesterone the time of onset of estrus could be controlled, if injections were started while a functional corpus luteum was present. Later Ulberg, Grummer, and Casida (1951) using gilts, and Ulberg, Christian, and Casida (1951) using heifers, were also able to control time of heat and ovulation by injecting sufficient levels

of progesterone, providing that injections were started before the animal's own supply of progesterone had decreased low enough to allow follicular growth. They found that for gilts 100 mg. of progesterone daily was required and for heifers 50 mg. daily was sufficient to inhibit heat and ovulation.

The data from these studies can be interpreted in accord with the hypothesis that progesterone inhibits or blocks the gonadotrophic complex. Evidence from a study of follicular size in animals under different levels of progesterone indicates that perhaps the luteinizing hormone is prevented from interacting with the follicle-stimulating hormone, since higher levels of progesterone result in smaller follicular size than threshold levels (Dutt and Casida, 1948).

An interesting observation from these studies relating to a possible cause of cystic follicles is noted. Dutt and Casida (1948) noted in ewes that levels of progesterone which did not completely suppress ovulation (5 mg. daily) resulted in follicles in ovaries of some treated ewes which appeared to be cystic in nature. In gilts it was found that injection of 50 mg. of progesterone daily was a dosage high enough to prevent heat and ovulation, but resulted in the formation of cystic follicles which persisted for at least a month after the treatment had stopped (Ulberg, Grummer and Casida, 1951). This condition was not noted if the dosage was either decreased or increased. It is postulated from this work that the production of cystic follicles is caused by critical low levels of luteinizing activity in relation to follicle-stimulating activity. Partial depression of luteinizing activity by low levels of progesterone results in overgrowth in size of follicles which are incapable of ovulation.

The action of progesterone in controlling the time of heat has been used in conjunction with pregnant mare serum in successfully inducing synchronous estrus and ovulation in anestrual ewes. There is evidence that the ovaries of anestrual ewes are not inactive during the greater part of the non-breeding season, but actually pass through rhythmic change (Cole and Miller, 1935; and Robinson, 1950). Injecting anestrual ewes with progesterone for a period of fifteen days prior to injecting pregnant mare serum resulted in all ewes showing synchronous estrus and ovulation one to two days later (Dutt, 1952). Shorter periods under progesterone treatment resulted in a decreased percentage of treated ewes showing heat, even though all of the ewes had ovulated. Pregnant mare serum alone resulted in all ewes ovulating, but none showed heat. It is proposed that synchronization of the ovaries in all treated anestrual ewes was effected by progesterone to a stage similar to that of the waning corpus luteum and, when the ovulating hormone is injected, synchronous estrus and ovulation occurs because all ewes are at a similar stage in a subthreshold cycle.

Ovulation-stimulating Effect of Progesterone

Progesterone, when administered at certain stages in the cycle, also appears to have a stimulating effect on estrus and ovulation in some species.

Dempsey, Hertz, and Young (1936) using spayed guinea pigs, and Boling and Blandau (1939) using spayed rats found that estrus could be produced in a higher percentage of animals if progesterone was injected

following estrogen, as compared to estrogen injection alone. Later Everett (1943) with a strain of persistent estrual rats was able to interrupt estrus and cause ovulation by injecting progesterone. In normal rats Everett (1948) found that progesterone injection on the third day of diestrus caused ovulation approximately 24 hours earlier than it normally occurs. Sawyer, Everett, and Markee (1950) injected estradiol benzoate, progesterone, or a combination of the two into estrual rabbits. Ovulation did not occur as a result of injecting either estrogen or progesterone alone, but did result from the combined treatment. Pfeiffer (1950) was able to produce ovulation in the monkey during the summer anovulatory period by progesterone injection. From these results it is inferred that progesterone is produced by the granulosa cells in the still-unruptured follicle, and that sexual receptivity and ovulation are the result of the synergistic action of estrogen and progesterone, and that release of the luteinizing hormone from the pituitary gland is the result of the action of progesterone secreted by the ovary.

In order to determine the effect of progesterone upon time of ovulation in farm animals, Hansel and Trimberger (1952) injected dairy heifers at the beginning of heat with progesterone and found that the interval from onset of heat to ovulation was 9 hours shorter than for control intervals on the same animals. When progesterone was injected four to five hours before the onset of heat, time of ovulation was also hastened, but when injection was delayed until 5 to 6.5 hours after the beginning of heat, the time of ovulation was the same as for control periods. These workers suggest that progesterone is produced in the ovary before ovulation and that it plays a role in luteinizing hormone release and ovulation in the cow.

Simpson (1952) studied the effect of estrogen and progesterone and a combination of the two hormones during the heat period on the time of ovulation in ewes. Hormones were injected near the onset of heat and four groups of six ewes each were studied. The average intervals from onset of estrus to ovulation for the various groups are shown in Table 2.

Injection of one milligram of estradiol benzoate caused a delay in time of ovulation. The average interval from onset of estrus to ovulation was 36 hours for the control ewes and 44 hours for the ewes receiving estradiol benzoate. Injecting progesterone hastened time of ovulation. The average interval from onset of estrus to ovulation for ewes injected with progesterone was 32 hours. Neither of these treated groups was significantly different from the control group in time of ovulation; however, the difference in time of ovulation between groups receiving estradiol benzoate and progesterone was statistically significant. When estradiol benzoate and progesterone were administered together to ewes, the average ovulation time was the same as that for control ewes.

These studies on heifers and ewes cast some doubt on the efficacy of the estrogens to cause release of the luteinizing hormone in these two species. In both species injection of estrogens at the onset of heat has resulted in a delay in ovulation time; whereas, injecting progesterone has resulted in hastening the time of ovulation.

The several-fold increase in duration of estrus in the ewes receiving estradiol benzoate indicates that the dosage was probably well above the physiological limit, and the effect of lower levels of estrogen on the time

of ovulation should be studied. The length of the estrual period reported for dairy heifers treated with estradiol indicates that the level of estradiol used was not greatly above that of the physiological level for cattle.

From these studies it is apparent that indiscriminate doses of estrogenic substances are of doubtful therapeutic value for inducing or regulating time of ovulation in the ewe or the cow. There is great need for more studies of this nature using different levels of estrogen. Also, time of treatment with regard to stage of the cycle in larger animals needs more critical study. These results should point out to us that perhaps we ought to take another look at our present concept of the mechanism for reciprocal relationship between the ovary and the pituitary gland.

TABLE 2

Hours from Onset of Estrus to Ovulation and Duration of Estrus for Ewes Injected with Estradiol Benzoate, Progesterone, and Estradiol Benzoate-Progesterone at Beginning of Heat. *

Treatment Groups	Control	1 mg. Estradiol Benzoate	30 mg. Progesterone	1 mg. Estradiol Benzoate and 30 mg. Progesterone
Number of Ewes	6	6	6	6
Mean Interval Onset Heat to Ovulation (hrs.)	36 ±2.5	44 ±4.7	32 ±2.7	36 ±2.5
Mean Duration of Heat (hrs.)	30±2.5	96 ±9.8	22±2.0	74±6.4 **

* From Simpson, E. C., Master's Thesis, University of Kentucky, 1952.
** Three of the ewes in this group showed split estrus.

The results reported on the effect of progesterone on time of ovulation have some interesting aspects. Does progesterone cause the pituitary gland to release luteinizing hormone, or is it merely a "rebound phenomenon"? That is, does the injection of progesterone temporarily block the release of luteinizing hormone, after which the sudden rise in level of luteinizing hormone is responsible for earlier ovulation? Masson and Hoffman (1945) have shown that progesterone is rapidly metabolized in the blood stream by the liver, which may be supporting evidence for accepting the theory of a temporary blocking followed by a sudden rise in the level of luteinizing hormone. Progesterone may have a direct effect on the pituitary gland through a synergistic effect with estrogen. It has been shown in the monkey (Salhanick, Hisaw, and Zarrow, 1952) that treatment with both estrogen and progesterone in moderate doses gave better depletion of the pituitary gland than did treatment with estradiol alone. Also,

Dempsey, Hertz, and Young (1936) advanced the hypothesis that estrus and ovulation are brought about by the immediate action of progesterone, which is elaborated in the pre-ovulatory follicle shortly before ovulation, and ovulation taking place as a result of release of luteinizing hormone by the pituitary gland. The generally accepted theory that the gonadotrophic hormones are mostly under control of the estrogens may need some revision in light of the more recent work with progesterone. Here again, more critical work needs to be done so that a better understanding of the ovarian-pituitary hormone relationship is had.

SUMMARY

The literature on the effect of estrogens and progesterone on time of ovulation in laboratory and farm animals has been reviewed.

Experimental evidence in laboratory animals supports the view that physiological levels of estrogen result in a suppression of follicle stimulating hormone and a release of the luteinizing hormone. The limited amount of critical information on the effect of estrogens on time of ovulation in farm animals does not show that they are effective in shortening the interval from onset of estrus to ovulation. When estrogens are injected near the onset of heat, ovulation time was delayed in both dairy heifers and ewes. Treating anestrual ewes with estrogens has also proven to be of little value in inducing ovulation and out of season breeding.

In both laboratory and farm animals progesterone has been shown to prevent ovulation and this action appears to be through a suppression of the release of the luteinizing hormone from the pituitary gland. Injecting progesterone in sufficient amounts, before the endogenous supply of progesterone has decreased, has been used as a means of controlling time of heat and ovulation in heifers, in gilts, and also in ewes during the breeding season. Progesterone has also been injected for a period of time prior to pregnant mare serum as a means of inducing synchronous estrus and ovulation in anestrual ewes.

Evidence has also been reviewed showing that injection of progesterone at the onset of heat hastens the time of ovulation in dairy heifers and ewes. There is little doubt that the secretion of the gonadotrophic hormones are regulated by the sex steroid hormones, but their specific role in regulating the pituitary gland is still questionable.

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ROLE OF THYROID HORMONE IN REPRODUCTIVE PHYSIOLOGY OF THE FEMALE*

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Although numerous investigations, conducted over a period of more than half a century, indicate that the thyroid hormone is implicated in some manner in the reproductive process of the female, the precise role that it plays has not been elucidated. An analysis of the information available to date indicates that there are nicely balanced interactions between the hormones of the pituitary, the ovary, and the thyroid. An excess or deficiency of one hormone or group of hormones will result, in most cases, in impaired reproductive function. It is the purpose of this report to review the influence of the thyroid hormone on ovarian function, with particular reference to the hormonal interrelationships that are involved. One aspect of this problem includes the effects on the estrous cycle.

Influence of the Thyroid Status on the Estrous Cycle

It is well established that either a deficiency or a large excess of thyroid hormone will cause aberrations in the estrous cycle. Evans and Long (1921a) reported that in rats thyroidectomy was usually followed by a pause in estrous cycles, but that this in turn was succeeded by normal cycles. More recent investigations indicate that either thyroidectomy (Lee, 1925; Richter, 1933; Freedman, Wright, and Webster, 1935; Nelson and Tobin, 1937; Ross 1938) or goitrogen administration (Mann, 1945; Jones, et al., 1946; Krohn and White, 1950; and others) will cause an increase in the length of the estrous cycle and will also increase the variability in cycle length.

Krohn (1947) found that daily subcutaneous administration of propylthiouracil disturbed the normal estrous rhythm of mature albino mice, causing lengthening, irregularity, or complete disappearance of the cycles.

On the other hand, Dragstedt, Sudan, and Phillips (1934) reported that complete thyroparathyroidectomy did not prevent the regular appearance of estrous in bitches. Brody and Frankenbach (1942) and Spielman, et al. (1945) observed that thyroidectomized cattle failed to show physical manifestations of estrous.

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It was reported by Engle (1944) and Aranow, Engle, and Sperry (1946) that rhesus monkeys that were either thyroidectomized or treated with thiouracil showed menstrual irregularity, with frequent occurrence of amenorrhea.

As pointed out by Foster and Thornton (1939), clinicians have repeatedly found thyroid therapy to be the most effective treatment for correction of menstrual abnormalities in women.

Although a certain level of thyroid hormone is essential for normal estrual behavior, an excess of this hormone will cause pronounced irregularities.

Gudernatsch (1915) reported that the onset of pregnancy was delayed in rats by feeding fresh thyroid tissue. Evans and Long (1921b) observed that the estrous cycle of rats was not greatly disturbed by feeding 0.25 to 0.5 gm. of fresh beef thyroid daily, but with larger doses the cycle was greatly lengthened or totally suppressed. Similarly, Drill, Overman, and Leathem (1943) reported that female rats fed 100 mg. of desiccated thyroid daily showed continuous diestrus. A similar prolongation of the estrus cycle in mice fed desiccated thyroid was reported by Cameron and Amies (1926).

The inhibition of estrus by thyroid feeding might be explained in part by the fact that the quantity of estrogenic hormone required to induce estrus in hyperthyroid rats is markedly increased (Reiss and Pereny, 1928; Van Horn, 1933; Langham and Gustavson, 1947; Hill, 1948). It appears, however, that the primary cause will be found in changes observed in the development and function of the ovary.

Influence of Thyroid Status on Ovarian Function

In hypothyroidism the ovarian picture is principally one of follicular growth. Hofmeister (1894) reported that the ovaries of thyroidectomized rabbits contained abnormally large follicles packed closely together. Tatum (1913) observed that the ovaries of cretin rabbits had fewer primary follicles than normal, an increase in size of follicles, and degenerative changes in the ova. These findings were confirmed by Kunde, Carlson, and Proud (1929). Similar changes in the ovaries of mice treated with goitrogens were reported by Dalton, et al. (1945) and Pawick (1947). Little or no effect on actual fertility of mice is noted if they are given the thiouracil for only two or three weeks before mating (Hurst and Turner, 1948; Ward, 1950). Long continued administration of goitrogens to rats, however, resulted in almost complete prevention of conception (Goldsmith, et al., 1945; Barker, 1949).

In hyperthyroidism it is usually observed that function of the lutein cells of the ovary predominates. Weichert and Boyd (1933) observed that rats fed 0.25 or 0.5 gm. of desiccated thyroid had one, two, or three estrous cycles followed by a long diestrous interval of 10 to 22 days. The ovaries contained large corpora lutea and the uteri formed placentomata when suitably stimulated, indicating that the rats were pseudopregnant. In rats raised to maturity on 0.5 and 1.0 per cent of desiccated thyroid in their diet (Ershoff, 1948) the ovaries remained infantile in both weight and histological appearance. On the other hand, Da Costa and Carlson (1933) observed that sexual maturation was accelerated slightly in rats fed 0.5 to 1.0 mg. of desiccated thyroid daily.

Kunde, Carlson, and Proud (1929) reported that severely hyperthyroid rabbits undergo estrus, ovulation, fertilization, and implantation; but fetal resorption occurs during the last one-third of pregnancy. Hyperthyroidism (Hurst and Turner, 1948) was detrimental to reproduction in mice, conception occurring in only seven out of ten animals. It should be emphasized that the foregoing results apply only to conditions of extreme hyperthyroidism.

Experiments were conducted in our laboratories (Ward, 1950) to determine the thyroxine tolerance range of female mice for normal reproduction. Experiment I included 25 multiparous mice of the Rockland strain, divided into groups of five. In Experiment II, 50 virgin Rockland female mice were divided into groups of 10. The schedule of thyroxine dosages is given in Table 1, together with data on number of conceptions and litter size. In each case daily thyroxine injections were given for two weeks, before placing male mice with the females, and continued during the gestation period. It was assumed that the normal thyroid secretion rate of the mouse is on the order of 5.5 µgm. per 100 gm. body weight (Hurst and Turner, 1948). Dosages of from one to eight times this amount were given in Experiment I and from one-half to four times in Experiment II. The highest dosage level in Experiment I was toxic, and the one surviving mouse failed to produce a litter. However, there was no significant difference in the number of mice conceiving or in the litter size between the controls and mice receiving thyroxine up to four times their normal secretion rate (Table 1). It is thus apparent that, despite the unfavorable results of pharmacological doses, there is a considerable range of thyroid function that is compatible with normal ovarian function and reproduction.

TABLE 1

The Effect of Varying Degrees of Hyperthyroidism on Number of Conceptions and Litter Size in Albino Mice*

Thyroxine Daily µgm. per 100 gm. Body Weight	Number of Mice	Number of Litters	Litter Size
Experiment I			
0	5	4	7.0 ± 1.3**
5.5	5	5	10.4 ± 1.4
11.0	5	3	11.0 ± 1.5
22.0	5	2	6.5 ± 1.5
44.0	5	0	- -
Experiment II			
0	10	5	8.0 ± 0.5
2.75	10	7	7.4 ± 0.5
5.5	10	6	6.7 ± 1.0
11.0	10	5	6.6 ± 1.3
22.0	10	6	9.8 ± 1.6

* Taken from Ward (1950).

** ± Standard error.

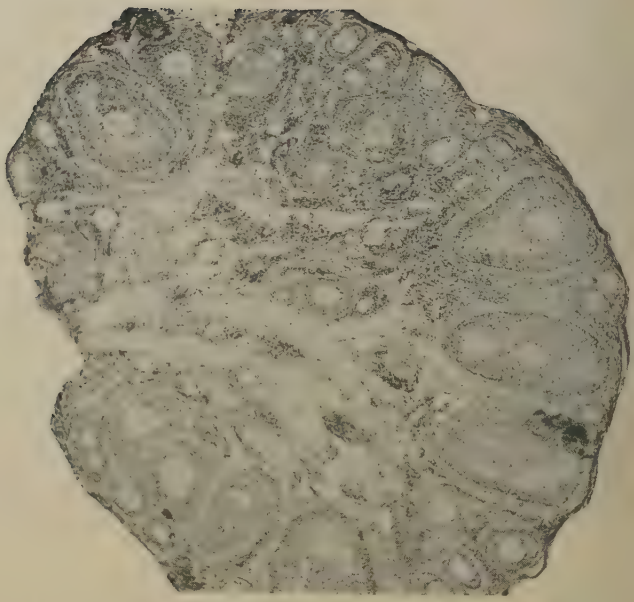

Fig. 1. Ovary section from normal mouse kept at 24° C. Note: (1) cortex with graafian follicles at different stages of development, (2) lack of corpora lutea.

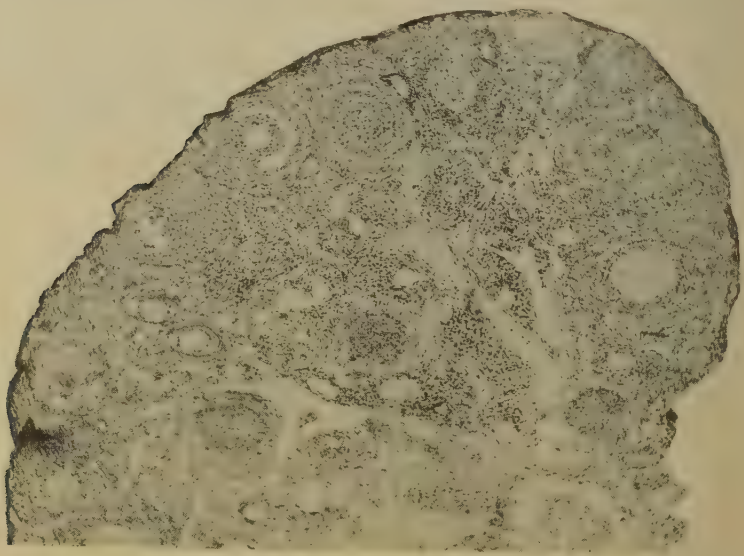

Fig. 2. Ovary section from mouse kept at 30° C. Note: (1) small size of follicles and (2) lack of corpora lutea.

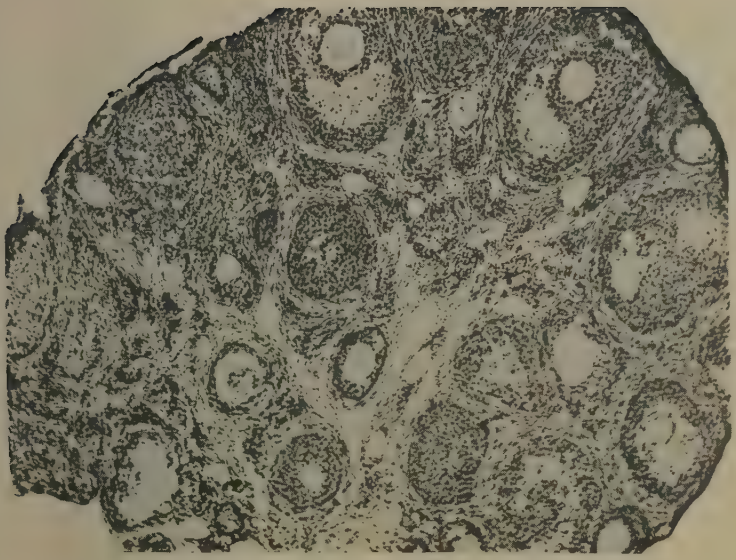

Fig. 3. Ovary section from mouse kept at 24° C. and given 0.1 per cent thiouracil in the ration. Note the large number and size of graafian follicles.

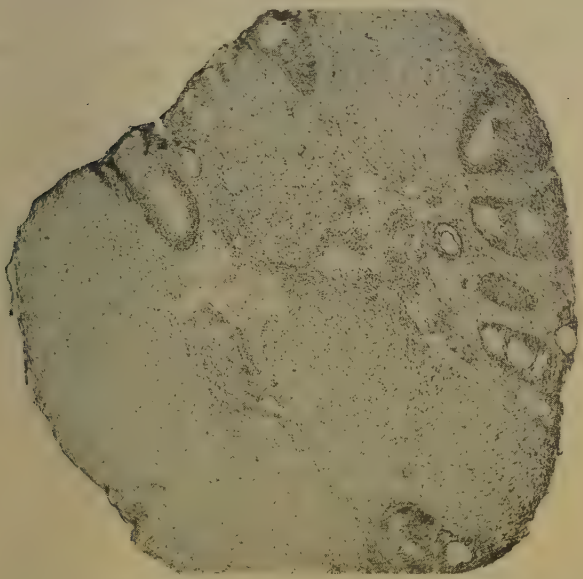

Fig. 4. Ovary of mouse kept at 24° C. and given 0.05 per cent thyroprotein in the ration. Note: (1) the extensive development of corpora lutea, (2) the presence of growing follicles.

The effects of both hypo- and slight hyperthyroidism on the ovary of the mouse are illustrated in an earlier report by the authors (Soliman and Reineke, 1952). In this work groups of immature female Rockland mice, with an initial body weight of 14-16 gm., were allocated to various groups maintained at 24°, 30°, and 35° C. Certain of the subgroups were given thiouracil or small amounts of thyroprotein incorporated in their diet. The animals were sacrificed after 4 weeks under these conditions, and data were collected on the condition of their reproductive organs.

In mice kept at the normal room temperature of 24° C. ovary weights were increased significantly by 0.0125 per cent thyroprotein, but depressed by higher levels. At 30° and 35° C., 0.005 per cent thyroprotein had no effect on the weight of the ovaries. However, thyroprotein at all environmental temperatures and all dosage levels employed caused significant increases above their controls in weight of the uterus. This is indirect evidence of increased hormone output by the ovary. Certain of the thiouracil-treated mice also had enlarged uteri, but this was found to be due to edema and accumulation of watery fluid in the uterine cavity. Study of vaginal smears showed these animals to be in continuous estrus, whereas the thyroprotein groups went through regular estrus cycles.

Histological examination of the ovaries revealed that the control mice kept at 24° C. (Fig. 1) had not yet reached full maturity, showing only follicles at various stages of development. High environmental temperature retarded ovarian development (Fig. 2). The ovaries of thiouracil-treated mice (Fig. 3) were packed with large follicles, but contained no corpora lutea. Ovaries of all thyroprotein-treated mice studied had numerous corpora lutea (Fig. 4) together with growing follicles. The histology of the uteri in the various groups was closely correlated with that of the ovaries. Controls had typically anestrous uteri with a thin wall, small uterine glands, and a low epithelium. Thiouracil-treated mice showed an estrous uterine histology with tall columnar epithelium, shrunken elliptical nuclei in the stromal cells, and pronounced edema. Progestational uteri, indicating functional corpora lutea, were found only in the thyroprotein groups. They contained a thickened endometrium and distinctly rounded stromal nuclei typical of progesterone stimulation. These results indicate that hypothyroidism favors follicular growth whereas hyperthyroidism favors luteinization. It was suggested further that there may be cyclical variations in thyroid function, correlated with changes in output of gonadotrophin by the pituitary. Suggestive evidence along this line is also available from direct assay of pituitary hormones.

Effect of the Thyroid Status on Pituitary Gonadotrophin

Considerable disagreement exists regarding the effect of thyroidectomy on the gonadotropic hormones content of the pituitary. Evans and Simpson (1930) thyroidectomized young female rats and after 5 weeks assayed their pituitaries in comparison with those from controls. The cretinous pituitaries were less effective than normals in inducing sexual maturity in young rats. Young adult female rats that were fed thyroid for five weeks had pituitaries that were more effective than the normals in evoking this response. In contradistinction to these results, Smith and Engle (1930) reported no change in AP gonadotrophin content of thyroidectomized female rats, and Smelser (1934) reported no change in male rats.

Turner and Cupps (1940) noted a pronounced decline in AP gonadotrophin content of thyroidectomized male, but not of similarly treated female rats.

A reduction of the gonadotropic potency of the pituitary following thyroidectomy was reported for normal and castrate rabbits and rats by P'an (1940), for young male goats by Reineke, Bergman, and Turner (1941), and for male rats by Stein and Lisle (1942). However, Okano and Tanaka (1940) concluded that thyroidectomy in the rabbit increases the pituitary gonadotrophin above normal.

Van Dyke and Chen (1933) found that the rabbit ovulation inducing factor was reduced, but not absent, in pituitaries of thyroidectomized rabbits in comparison with litter mate controls. Chu (1944) reported that whereas pituitaries of normal rabbits caused 60 per cent ovulation in estrous females, pituitary extracts from thyroidectomized rabbits failed to cause ovulation but instead caused increased growth of follicles. Furthermore, thyroidectomized females failed to ovulate when injected with extracts from thyroidectomized donors, but responded readily to normal AP extracts. He also observed that despite the presence of an increased number of follicles in the ovaries of thyroidectomized rabbits, ovulation does not occur after coitus or after the injection of copper salts. The failure to ovulate after coitus was confirmed by Fredrickson and Rydin (1947).

If the results cited above are correct, one would expect complete reproductive failure in the thyroidectomized rabbit due to arrested ovulation. Yet, Sachs (1939) reported that ovulation, fertilization, and development of the ovum are normal in the thyroidectomized rabbit, but the advanced embryo dies. Recently Krohn (1951) stated that pregnancy and parturition can follow a normal course in such animals.

The reason for these divergent findings is not apparent. In view of the difficulty of producing a complete thyroxine deficiency in thyroidectomized animals, however, it appears possible that results of the type reviewed might be modified by the iodine content of the diet, the ingestion of traces of thyroidal substances, or various environmental factors. The influence of dietary iodine and thyroxine and a favorable environmental temperature in counteracting some of the effects of thyroidectomy in the rat has been demonstrated by Leblond and Eartly (1952).

Effect of Gonadectomy on Thyroid Function

There is little agreement regarding the effect of gonadectomy on thyroid function in guinea pigs. Chouke (1930) and Starr and Bruner (1935) found no change in thyroid histology of female guinea pigs subsequent to castration, Loeser (1934) reported an increase of AP thyrotropin in such animals, and Kibben and Loeb (1936) reported an increase in mitotic index of the thyroid.

In the rat, Anderson and Kennedy (1933) found no changes in thyroid histology one week after castration, but reported definite atrophy in thyroids of both males and females castrated eight weeks or more. Emge and Laquer (1941) also reported histological evidence of reduced thyroid function in female rats after castration. Turner and Cupps (1940) reported that the thyrotropin of the anterior pituitary of both male and female rats was reduced slightly 20 days and markedly 66 days after

castration. Hurst and Turner (1948) reported a reduced thyroid secretion rate of male mice 16 weeks after castration. It is of interest that a reduction of thyrotropin of the pituitaries of steers as compared to bulls has been reported by Bates, Riddle and Lahr (1935) and Reece and Turner (1937).

Modification by Thyroid of Response to Gonadotrophin

Fluhmann (1934) noted that the ability of pituitary gonadotrophin to increase ovary weight in the rat was reduced by contamination with thyrotropic hormone. Thyroidectomy increased and thyroid feeding decreased the response. Leonard (1936) reported that pituitary FSH produced a greater effect on ovaries and uteri of thyroidectomized than of normal rats, whereas pregnancy urine produces an equal effect in both types of rats. Therefore, it was concluded that thyroid hormone prevents or inhibits the action of FSH but not of LH. Johnson and Meites (1950) observed that in rats hyperthyroidism reduced the ovarian weight increase obtained by injecting pregnant mares' serum. Short term thiouracil feeding (4, 7, 10, and 15 days) increased the response, but it was reduced in rats given thiouracil for 20 days. In young mice, on the other hand, the ovarian response was increased by hyperthyroidism but reduced by hypothyroidism.

Variations in Thyroid Function During the Estrus Cycle

Until recently little information has been available on variations in thyroid function during the estrus cycle.

Lee (1927, 1928) reported a significant increase in the basal metabolic rate of female rats during the last 10 hours of diestrum and the first 6 hours of proestrum. Hunt (1944) observed that in young female rats the mitotic activity of the thyroid, increased somewhat in early estrus, was maximal during estrus, declined during metestrus, and was minimal during the diestrous interval.

Further experiments were undertaken by the authors in an attempt to clarify these relationships (Soliman, 1952; Reineke and Soliman, 1953). Since the metabolic rate is usually considered to provide a relative measure of thyroid activity, daily metabolism measurements were taken on female rats and the values were compared with the stage of the estrus cycle, as determined by vaginal smears.

Oxygen consumption was determined by the authors' modification of the manometric technic of MacLagan and Sheahan (1950). The rats were fasted 12 to 15 hours prior to each measurement, and vaginal smears were taken daily. Data were obtained on three groups of 12 rats each (Carworth strain). Eight determinations were made on consecutive days on the first group, 15 on the second, and 10 on the third. This comprises a total of 396 oxygen consumption values and an equal number of vaginal smears. A summary of the results shows a quite uniform oxygen consumption, averaging 102.6 ± 1.67 ml. per 100 gm. body weight per hour during metestrus, 103.4 ± 1.17 ml. at diestrus, and 104.1 ± 1.49 ml. at proestrus. During estrus, the oxygen consumption showed a statistically significant rise (one per cent level) to 120.6 ± 2.0 ml. This rise came slightly later in the cycle than reported by Lee (1928), but was similar in magnitude.

Although the elevation of oxygen consumption during estrus might be taken as presumptive evidence of an increase in thyroid function, the possibility remained that such a rise could occur due to the increased physical activity of rats at this stage. It thus seemed desirable to test this finding by use of a more direct measure of thyroid function, and it was decided to do thyroid uptake studies with radioactive iodine (I^{131}).

Thirty-five mature female rats of the Carworth strain, weighing 250 to 270 gm. were used in the first experiment. They received a stock diet and drinking water ad libitum. To establish the estrual pattern of each rat, vaginal smears were taken daily for three days and every six hours for two days. Each animal was then injected, intraperitoneally with a tracer dose of I^{131}. Each rat was killed exactly six hours after the injection. The thyroids were removed, weighed, and prepared for counting. Radioactivity counts were made with a Geiger counter connected to a laboratory scaler. The ovaries were examined with a magnifying lens, and the uteri were weighed to confirm the stage of the estrus cycle obtained from the vaginal smears.

Inasmuch as the rats received a stock diet containing iodized salt in this experiment, it seemed conceivable that variations in dietary iodine intake might change the specific activity of the iodine in the blood sufficiently to account for observed changes in thyroid uptake. Therefore, a second experiment was run in which iodine intake was rigidly controlled. For 15 days prior to the experiment the rats were fed the Remington low-iodine test diet, given double distilled drinking water, and injected daily with 5 µgm. of NaI dissolved in distilled water.

The iodine uptake by the thyroid observed at different stages of estrus in both experiments is summarized in Table 2. It will be noted that in

TABLE 2

Six Hour I^{131} Uptake by the Thyroid Glands of Female Rats.

	Proestrus	Estrus	Metestrus	Diestrus
Experiment I				
Number of rats	7	16	6	6
Per cent Uptake of Injected Dose	11.16 ± 0.86*	17.48 ± 0.73	12.94 ± 1.64	11.76 ± 0.95
Experiment II				
Number of rats	13	8	14	8
Per cent Uptake of Injected Dose	8.17 ± 0.64	16.23 ± 0.65	10.79 ± 0.67	8.99 ± 0.78

* ± Standard error.

both experiments there is a significantly greater collection of iodine by the thyroid of the rat during estrus than in any of the other stages. This is followed by a progressive decline in iodine collection during metestrus and diestrus, with minimal levels being reached at proestrus.

It is of interest to note that in parallel experiments on mice a similar pattern of iodine collection by the thyroid was observed. However, the rise in uptake started during diestrus and reached the peak during proestrus rather than during estrus.

The data obtained demonstrate that both the metabolism and thyroid iodine collection are at their maximum in the female rat during estrus. These data, together with the findings reviewed earlier, suggest that the thyroid gland undergoes cyclic variations in function that are closely correlated with the hormonal changes in the pituitary and ovary.

Influence of Ovarian Hormones on Thyroidal I^{131} Uptake

To test this hypothesis further physiological doses of estrogen and progesterone were injected into ovariectomized rats, alone or in combination, in the sequence believed to occur during the estrous cycle, and the uptake of I^{131} by the thyroid was determined. Beginning five days after ovariectomy, the rats were given a controlled iodine intake as in the preceding experiment for ten days, and then divided into groups of six or seven animals for the various treatments. Group I was kept as control. Groups II, III, and IV were given a priming dose of two rat-units of estradiol benzoate one day before the designated treatments. The rats in Group II were then injected intraperitoneally with six rat-units of estradiol benzoate 48 hours before autopsy. Groups III and IV were injected with six rat-units of estradiol benzoate 72 hours before being sacrificed, and on the next day with 0.4 and 0.8 mg. of progesterone, respectively. The animals in Groups V and VI were injected with 0.4 and 0.8 mg. of progesterone 48 hours before autopsy. All rats were injected intraperitoneally with 1.0 microcurie of I^{131} six hours before being sacrificed.

The average I^{131} uptake by the thyroids of the various groups, expressed as per cent of injected dose, was as follows:

Group I, control	13.9 ± 0.47
Group II, 6 r.u. estradiol benzoate	16.8 ± 0.87
Group III, 6 r.u. estradiol benzoate + 0.4 mg. progesterone	11.5 ± 0.56
Group IV, 6 r.u. estradiol benzoate + 0.8 mg. progesterone	13.2 ± 0.98
Group V, 0.4 mg. progesterone	10.3 ± 0.75
Group VI, 0.8 mg. progesterone	15.2 ± 0.68

Thyroidal I^{131} uptake was increased significantly by six r.u. of estradiol benzoate only, but was depressed to control values or below when the estrogen was followed 24 hours later by either 0.4 or 0.8 mg. of progesterone. Progesterone alone depressed thyroid iodine uptake at the 0.4 mg., but significantly increased it at the 0.8 mg. level.

The sequence of estrogen and progesterone injections produced variations in thyroidal I^{131} uptake that closely parallel the changes actually

observed during the estrous cycle. In our opinion they strongly suggest that rhythmic alterations in thyroid function during the estrous cycle are mediated by changes in the levels and proportions of estrogen and progesterone secreted by the ovary.

The stimulating effect on thyroid iodine collection of a moderate single dose of six r.u. of estradiol benzoate has been repeatedly confirmed in a series of four different experiments on ovariectomized rats. Chronic estrogen administration at a higher level (a total of 300 r.u. given during 20 days) had no significant effect. This confirms the report of Paschkis, Cantarow, and Peacock (1948) who found that estradiol benzoate had no effect on I^{131} uptake when 50 μgm. was injected daily for 11, 12, or 13 days. Hurst and Turner (1948) reported that female mice receiving 0.3 mg. of dianiysylhexene per kg. of feed had a thyroid secretion rate of 3.6 μgm. d,l-thyroxine daily compared to the control value of 2.1 μgm. Wolterink et al. (1950) observed in intact female mice and rats that a low dose of estrogen given for three days stimulated, but higher dosages depressed, thyroidal I^{131} output. Money et al. (1950, 1951) reported that in male rats 50 μgm. estradiol injected daily for 10 days increased I^{131} uptake, but dosages of 1 mg. or 50 mg. daily had no effect. Engstrom et al. (1952) reported that estrogen administration caused a rise in serum precipitable iodine in both men and women.

Mode of Action of Ovarian Hormones in Influencing Thyroid Function

In view of the relationships observed thus far, it was of interest to determine whether the stimulating effect of estrogen and the depressing effect of progesterone on thyroid function is exerted directly on the gland or indirectly through one of the other endocrine glands.

It has frequently been observed that estrogen administration is followed by an increase in weight of the adrenal, though information on the effects of cortical hormones on thyroid function is equivocal Halmi et al. 1953 and others). Consequently, experiments similar to those already described were conducted on ovariectomized and ovariectomized-adrenalectomized rats.

In brief, the thyroidal I^{131} uptake of adrenalectomized-ovariectomized rats was depressed significantly below that of ovariectomized controls. The administration of 6 r.u. of estradiol benzoate to the doubly operated animals resulted, 28 hours later, in a significant increase in I^{131} uptake above that of either ovariectomized controls or ovariectomized rats receiving the same dose of estrogen. This demonstrates clearly that the thyroid stimulating action of estrogen is not dependent on the presence of the adrenals.

Finally, experiments were devised to test the role of the pituitary gland in the gonad-thyroid relationship. For this purpose the six-hour I^{131} uptake of the thyroids of hypophysectomized-ovariectomized and ovariectomized rats was compared by methods similar to those employed in the preceding experiments.

As expected, the I^{131} uptake was greatly reduced in hypophysectomized-ovariectomized rats as compared to ovariectomized controls. Expressed as per cent of injected dose, the former had an average of 0.80 ± 0.14 compared to 9.95 ± 1.45 for the controls. Forty-eight hours after injecting

either 6 or 300 r.u. of estradiol benzoate there was no significant change in the thyroid I^{131} uptake of the doubly operated animals, although similar treatment in ovariectomized animals again caused a significant rise.

A single dose of 0.2 mg. of progesterone did not significantly affect I^{131} collection, but 0.4 mg. caused a small but statistically significant decrease.

From these results it is apparent that estrogen exerts its effect on the thyroid via the pituitary gland, probably by influencing the secretion of thyrotropic hormone. It is tentatively suggested that progesterone acts through other pathways, but whether this is by direct action on the thyroid mechanism or through some other route cannot be stated at the present time.

Discussion and Summary

The preponderance of information now available indicates that there is a reciprocal balance between the hormones of the pituitary, the ovary, and the thyroid. Through the influence of estrogen on pituitary thyrotropin, the thyroid undergoes rhythmic fluctuations in secretion rate that in turn regulate the output of gonadotrophins and also modify their action on the ovary. A rhythmic sequence of changes apparently occurs in this balance during follicular growth, estrus, and ovulation. An imbalance in this hormonal mechanism will not necessarily result in complete reproductive failure, but usually leads to great irregularity.

Either hyper- or hypothyroidism results in lengthening or cessation of the estrus cycle in rats and mice, and amenorrhea in primates.

Thyroidectomy has been shown to cause a reduction in the gonadotropic hormone of the pituitary. Conversely, gonadectomy results in a decrease of pituitary thyrotropin. Results in rats, mice, and rabbits indicate that pituitary FSH action is favored by hypothyroidism, but LH action is favored by hyperthyroidism.

The metabolic rate, at least in rats, is increased significantly at estrus, compared to other stages of the cycle. Histologically, the thyroid also appears to be at the point of maximum activity at this stage.

Tracer studies with I^{131} show that the iodine uptake of the thyroid is at a maximum during estrus, decreases progressively during metestrus and diestrus and reaches a minimum during proestrus.

Experiments in ovariectomized rats show that thyroid I^{131} uptake is increased by a small, short term dose of estrogen. Larger dosages given for long periods have no effect. I^{131} uptake is depressed by progesterone. The adrenal glands are not needed for the effect of estrogen on I^{131} uptake to be expressed. Trials on hypophysectomized animals show that this action of estrogen is exerted via the pituitary. Progesterone appears to operate through some other pathway.

So far as the authors are aware, no effective applications have been made of the findings reviewed here in the regulation of reproduction in domestic livestock. Kammlade, Welch, Nalbandov, and Norton (1952) observed that the total gonadotrophin of the pituitaries of anestrus ewes is as high as that found during the breeding season. The ovaries also contain large follicles. They suggest that the failure to ovulate may be due to an imbalance between FSH and LH. Recently, Henneman (1953) has

shown that the thyroid secretion rate of two-year old ewes during July (anestrus season) is only about one-fourth as high as in the winter. Although these findings are very suggestive, it remains for further investigation to determine whether the anestrual condition can be corrected or modified by properly timed thyroidal stimulation.

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EGG TRANSFER AND SUPEROVULATION IN FARM ANIMALS

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The development of artificial insemination in the United States has been phenomenal. As a result, the dairy industry of the country stands to gain as it has from no other practice. The chief factor in the success of this enterprise has been the ability of the bull to produce billions of spermatozoa per ejaculate, thus enabling the dilution of semen to high levels and the breeding of thousands of cows per year to outstanding individual bulls. This success in the exploitation of the reproductive powers of the bull has stimulated interest in the possibilities of similarly exploiting the cow in order to obtain more offspring per year from outstanding females than is possible under customary methods of management.

Superovulation and egg transplantation have been suggested as the means to this end. The general procedure would be as follows: (a) administration of gonadotrophins to cause the production of many eggs at one time -- superovulation; (b) fertilization of the eggs in vivo or in vitro; (c) removal of the eggs from the cow; (d) holding the eggs for a short or a long period of time in vitro; (e) placing the eggs in the reproductive tract of the recipient.

Superovulation and egg transplantation are not new. Engle (1927b) first demonstrated with mice that superovulation could be accomplished with gonadotrophins. Superovulation was first accomplished in farm animals by Casida et al. (1943).

It was first demonstrated by Heape (1890) that young would develop from eggs transplanted from one rabbit doe to another. Biedl et al. (1922) were the first to verify Heape's work.

The first to produce living young in farm animals by transplantation of eggs were Warwick et al. (1934, 1949). Later Casida et al. (1944) recovered transplanted embryos in ewes up to 46 days after transplantation. Willett et al. (1951, 1953) have produced three calves by transplanting bovine eggs.

This paper attempts to outline the progress that has been made in the techniques for superovulation and egg transplantation, the problems involved and the outlook. Much of the discussion concerns cattle because much more of the research aimed at the development of these techniques has been done with this species than with other farm animals. Considerable reference is made to laboratory animals because work with them, not having been duplicated in larger species, serves to explain some of the phenomena observed, to suggest work for the future, and to indicate what might be accomplished.

SUPEROVULATION

To superovulate a cow or another mammalian female, both of the gonadotrophic hormones are required, the follicle-stimulating hormone (FSH) to bring about the maturation of a number of follicles and the luteinizing hormone (LH) to cause ovulation. FSH is usually injected subcutaneously. LH can be provided exogenously by intravenous injection or endogenously by timing the injection of FSH to have mature follicles present at estrus. The follicles are then ruptured by LH from the cow's own pituitary.

The several methods of producing superovulation which have apparently given results that were at least fairly reliable and satisfactory include: (a) subcutaneous injection of pregnant mare serum (PMS) during the middle of the cycle and at the same time expression of the corpus luteum (Brock and Rowson, 1952; Dowling, 1949; Hammond, 1949a; Hammond and Bhattacharya, 1944; Rowson, 1951). The cow will then come in heat two to four days later with most on the third day (Dowling, 1949). (b) Subcutaneous injection of PMS during the follicular phase of the estrual cycle and about four days before heat is due (Dowling, 1949 ; Hammong, 1949a; Hammond and Bhattacharya, 1944). (c) Subcutaneous injection of horse pituitary extract for three consecutive days starting six days before estrus is due (Dowling, 1949). (d) Sheep pituitary FSH injected subcutaneously for five consecutive days starting five days before estrus is due and on the sixth day unfractionated sheep gonadotrophin (USG) or human chorionic gonadotrophin (HCG) injected intravenously (Casida et al., 1943; Willett, et al., 1952a). (e) A single subcutaneous injection of PMS administered five days before estrus is due and on the sixth day USG intravenously (Willett et al., 1952a). (f) A single subcutaneous injection of PMS administered five days before estrus is due and on the sixth day HCG intravenously (Laing, 1945; Willett et al., 1952a; Brock and Rowson, 1952). The latter have compared different periods of time between injection of PMS and HCG. The greatest number of ovulations and percentage of follicles ovulated resulted when the interval was 5 to 11 days.

Brock and Rowson (1952) also compared endogenous LH alone with supplementation with exogenous LH in HCG following PMS when the corpus luteum was absent. HCG almost doubled the percentage of follicles ovulating. Willett et al. (1952a) found that USG produced approximately twice as many corpora as HCG when they were used in combination with either sheep pituitary FSH or PMS.

PMS has a tendency to produce large, unovulated follicles with high dosages. This has been observed by Folley and Malpress (1944), Willett et al. (1952a) and Dowling (1949) with cows and by other workers with other species. Willett et al. noted this when PMS was followed by USG but not when followed by HCG. Pincus (1940) noted with rabbits that PMS had a narrower dosage range than sheep pituitary gonadotrophins and that large "cystic" follicles and unruptured blood follicles were produced with large dosages. Cole (1936) suggested that these large follicles may be due to over-stimulation of the ovaries and the resulting rapid thickening of the follicle walls, thus preventing ovulation. The presence of LH in PMS may aggravate this condition.

Boyarsky et al. (1947) with estrus rabbits obtained more ovulations when injecting progesterone than with the controls. It was suggested that

progesterone might enhance release of FSH from the pituitary. In the cow, Rowson (1951) obtained a significantly larger number of ovulations with PMS and HCG with the corpus luteum present than with it absent as a result of his expressing the corpus from the ovary manually through the vaginal wall. He explained his differences by suggesting that, in the absence of the corpus, the early maturing follicles rupture, corpora develop and secrete progesterone, which prevents further ovulations. With an active corpus present, ovulations are prevented until more follicles reach maturity. Contrariwise, Willett et al. (1952a) had larger numbers with an inactive corpus during the follicular phase of the cycle than with the corpus present during the luteal phase. The supposedly inactive corpus may still be secreting progesterone during the follicular phase of the cycle. In any event, conditions during normal estrus may be somewhat different than during an estrus following expressing of the corpus, as has been suggested by Rowson.

With the first three methods of superovulation listed above, the cow is inseminated when she comes in heat. With the latter three treatments the cow is inseminated at the time of the intravenous injection. Umbaugh (1949) and Willett et al. (1952a) also inseminated the cow again the day following to insure sufficient spermatozoa at time of ovulation

With HCG or USG injected intravenously or with endogenous LH supplied by the cow's own pituitary at time of heat, the follicles ovulate within a fairly short time. Casida (1938) stated that ovulation occurs within 36 to 48 hours after the intravenous injection.

When one depends completely upon endogenous LH produced by the cow at estrus to ovulate the follicles, he can be certain that ovulation will take place on a certain day. Dowling (1949) reported that, following enucleation of the corpus luteum, estrus occurs in most cases from two to five days later with most occurring on the third day. Eighty-four per cent of normal cycles of cows are between 18 and 24 days in length (Asdell, 1946). It is the writer's opinion that controlled timing of superovulation, which is important when eggs from superovulated cows are to be used for transplantation, can best be obtained by injecting LH intravenously rather than by depending upon endogenous LH.

Willett et al. (1952b) demonstrated with dairy cows that number of corpora lutea decreased with successive superovulations. After three or four treatments the number of corpora was little greater in most instances than if no gonadotrophin had been administered. Whether this apparent refractoriness is due to antigonadotrophins, damage to the ovary, exhaustion of primary follicles, or to other causes is still to be determined.

Cows can be superovulated at any stage of the cycle (Casida et al., 1943; Folley and Malpress, 1944; Rowson, 1951; Zavodovskii, 1935; Willett et al., 1952a) or during pregnancy without apparent injury to the fetus (Casida et al., 1943).

In studies of superovulation with possible application to egg transplantation, the objective is to find ways of producing the maximum number of normal, fertilizable eggs. For this reason, one of the criteria of ovarian response to gonadotrophins would be number of corpora lutea. It might be well to keep in mind, however, that this criterion may not be a completely accurate indication of number of ova produced. Engle (1927a) examined 100 mouse ovaries and found 2 triovular and 16 biovular

follicles. Fekete (1950) likewise examined ovaries of 8 inbred strains of mice. Polyovular follicles were infrequent in all strains except one where, of 20 ovaries studied, there were an average of 6.1 polyovular follicles per ovary. Davis and Hall (1950) also found polyovular and anovular follicles in Norway rats. Research is needed to determine whether superovulation might influence the number of such follicles. Furthermore, Casida et al. (1943), Folley and Malpress (1944) and others have published data which indicate that ovarian size is not a satisfactory measure of response to gonadotrophins.

FERTILITY OF SUPEROVULATED EGGS

As indicated in the discussion above, superovulation can be produced in a variety of ways and during all stages of the estrual cycle and even during pregnancy. As a means of producing eggs in quantities for transplantation, however, it is not sufficient to produce numbers of eggs. The eggs must be fertilized and with our present state of knowledge, this must be done in vivo. Pincus (1939) reported fertilizing rabbit eggs in vitro. Other workers have not been able to do this successfully.

When transplanting an egg, the investigator should know whether or not it has been fertilized and is capable of normal development. Various criteria have been employed for evaluating fertility of mammalian eggs. Examination of eggs with a microscope to establish presence or absence of blastomeres approximately equal in size has been the most common method. Austin (1949b) and Chang (1950c) have, however, demonstrated that degenerating eggs can have fragments approximately equal in size. Austin suggested the phase-contrast microscope as a means of distinguishing between fragments and blastomeres. Austin and Smiles (1948) and Austin (1951) have also devised methods for examining with a phase-contrast microscope for presence or absence of male and female pronuclei in one-celled eggs and thereby determining whether such eggs have been fertilized. His work was done with rats. Eggs of most farm animals have considerably more fatty globules in their cytoplasm than the rat (Boyd and Hamilton, 1952), and their nuclei may not be observable in this way. Differentiation of live and dead ova by stains has been studied by Brock and Rowson (1952). The only completely reliable method of establishing whether an egg has been fertilized and is capable of developing normally is to place it in an environment which allows normal development -- the uterus.

Runner (1951) in work with mice and Noyes (1952) with rats have transplanted unfertilized eggs into females previously mated. Fertilization took place in the recipients. They differentiated between young, developing from the donors' transplanted eggs, and those from the recipients' own eggs by genetic markers. In a dairy cow, where multiple births are undesirable (Pfau et al., 1948), both the donor's and the recipient's eggs would likely develop. Multiple births may, on the other hand, be desirable in beef cattle and are definitely desired in swine and sheep.

Are superovulated eggs capable of fertilization and normal development? Warwick et al. (1943) transplanted superovulated and normally ovulated rabbit eggs and obtained similar survival of the embryos. The numbers involved were small, however. By superovulating rabbits,

Chang (1948b) recovered 53 eggs from one doe, transplanted them into 4 does, and obtained 45 normal young. Thirty-five eggs were obtained from another female, and 26 young were obtained from 3 recipients. These data suggest that superovulated ova are capable of fertilization and normal development. More data are, however, needed to establish this point.

Dowling (1949) and Umbaugh (1949) have observed a rapid passage of eggs down the fallopian tubes in cows whose ovaries were stimulated excessively with PMS. Dowling made these observations when cows were superovulated with PMS following expression of corpora and Umbaugh with USG in pellets placed subcutaneously and followed by USG intravenously. Whitney and Burdick (1938) observed rapid egg travel down oviducts in rats given massive doses of estrogen. With excessive stimulation estrogen could be produced by the large, unovulated follicles in large quantities. Rowson (1951) also noted rapid passage of eggs when cows were superovulated with PMS and HCG in presence of the corpus luteum. It is well established that progesterone accelerates egg transportation (Chang and Pincus, 1951). Lamming and Rowson (1952) mentioned, however, that exogenous progesterone did not increase speed of travel of eggs in rabbits and suggested that this phenomenon must be due to some factor other than progesterone in the corpus.

As mentioned previously, it is characteristic of PMS to produce large follicles when excessive doses are given. Under these conditions fertilization rates are low (Dowling, 1949; Willett et al., 1952a). Dowling, however, had satisfactory fertilization rates in cows with horse pituitary extracts in the follicular phase of the cycle. Willett et al. observed marked superovulation (up to 55 corpora in a pair of cow ovaries) following administration of highly purified sheep pituitary FSH and USG and 74 per cent cleavage rates. These workers and also Brock and Rowson (1952) have presented evidence indicating that satisfactory fertilization rates of cow ova can be obtained with PMS and HCG. The latter have also shown that an interval of five days between injection of PMS and of HCG is optimum for production of large numbers of fertilizable eggs. Longer intervals caused degeneration of eggs.

As has been pointed out earlier, cows can be superovulated at any stage of the cycle. It has, however, been amply demonstrated (Casida et al., 1943; Rowson, 1951; Zavodovskii et al., 1935; Willett et al., 1952a) that, when a cow is superovulated and inseminated in the presence of a functioning corpus luteum, the eggs are not fertilized. Tanabe et al. (1949) also noted this in the sow, and Murphree et al. (1944) in sheep. Murphree et al. (1951) and Black et al. (1951) largely overcame this difficulty in rabbits by inseminating in the uterus. They concluded that infertility was due mainly to poor spermatozoan transport through the cervix. Austin (1949a) reached the same conclusion. Black et al. (1951) have demonstrated, by reciprocal transplantations between estrual and pseudopregnant rabbit does, that the eggs are fertilizable and capable of normal development. Inadequate sperm transport is not the complete explanation in the cow, for Willett et al. (1952a) obtained no fertilized eggs during the luteal phase even though the cows were inseminated in the uterus.

Brock and Rowson (1952), with an active corpus luteum present,

followed PMS with estrogen for one or more days before administering LH and inseminating the cows. Some of the cows showed heat, but there was no fertilization.

There is a possibility that only infertile eggs may be obtained when a cow is superovulated during the follicular phase, if the cow comes in heat a few days before LH is given intravenously, and if the cow is inseminated at the time of the intravenous injection. Corpora developing from follicles ruptured by endogenous LH will be present at the time of insemination and of ovulation by exogenous LH. Willett et al. (1953) observed this situation in heifers when progesterone was injected daily (Ulberg et al., 1951) prior to superovulation to control the time of ovulation and thereby to synchronize superovulation in the donor with estrus in the recipient.

Folley and Malpress (1944) observed a "shock" effect in ovaries of cows injected subcutaneously with horse pituitary extract. Single or double ovulations occurred within one or two days after the single injection and before follicular development by the hormone could have taken place. These workers attributed this condition to the presence of a well-developed follicle which was ruptured prematurely by the "shock" effect of the sudden impact of exogenous gonadotrophins, especially LH. Six cases were observed during the middle of the cycle when no normal ovulation was expected. Willett (1953) has observed a similar condition when superovulating cows in the follicular phase with sheep pituitary FSH and USG. A single, well-developed corpus luteum was present in addition to a number of younger corpora apparently developed by gonadotrophin. Eggs were not fertilized, and pus was usually present in the uterus. This condition might be explained by "shock" effect of gonadotrophins or by the possibility that estrus occurred earlier than expected. The normally developed single follicle may have been ruptured by the endogenous LH. This follicle had developed into a functioning corpus luteum by the time of the multiple ovulations produced by the injections.

EXTRACTION OF EGGS FROM FEMALE

Most of the research with mammalian eggs has involved slaughter of the donor, dissecting out the reproductive organs and flushing out of the eggs. Allen et al. (1930) with the human, Avis and Sawin (1951), Chang (1952), and Venge (1952) with the rabbit, and Umbaugh (1949) with the cow have extracted eggs from the living female by entering the abdominal cavity by laparotomy and flushing the eggs from the reproductive tract with the organs in situ.

Dracy and Petersen (1950) have attempted to make the ovary more accessible for procuring eggs by transplanting it to the paralumbar cavity, subcutaneously in the neck, or to the vagina. They also attempted to exteriorize the cut end of the uterine horn. None of these methods was satisfactory. These workers also attempted to develop a more practical solution to the problem by inserting a metal tube through the vagina and cervix and into the uterus and inserting through this tube a flexible plastic tubing which was inserted toward the anterior end of the uterine horn. Physiological saline solution was pumped into the uterus through the flexible plastic tubing and drained out between the flexible tubing and the metal tubing. In 37 trials they were able to recover eggs 12 times. When

the writer attempted to use this method in a number of trials, considerable difficulty was encountered with trauma and the presence of considerable quantities of blood in the washings.

A more satisfactory instrument for this purpose has been developed by Rowson and Dowling (1949). This apparatus consisted of a triple-lumen tube. One lumen permitted entrance of a steel rod to hold the tube rigid during insertion through the cervix. After the instrument was in place, the rod was removed and this lumen was used for removal of liquid from the uterus. Another lumen was to introduce liquid into the uterus. A third lumen enabled the forcing of air into a rubber collar several inches back from the tip of the instrument. This collar was inflated to hold the instrument in place and to block the horn to prevent escape of liquid. Rowson and Dowling reported recovering some eggs with this apparatus, although the instrument apparently has not been completely satisfactory. A modification of this instrument has enabled Donker (1953) to extract eggs from the cow with fair success. With additional refinement of equipment and with the development of skill by the operator, a practical means of recovering eggs from the cow appears possible.

EGGS IN VITRO

Whether eggs are to be held in vitro for a few minutes or for many hours, a satisfactory medium is essential. Various preparations have been used by workers who made successful transplantations. These include, to list only a few, Kreb's solution with rabbits (Black et al., 1951); phosphate buffered Ringer-Dale solution (containing 0.1 per cent glucose at pH 6.5 - 7 with an equal volume of fresh rabbit blood and then centrifuged) with rabbits (Chang, 1952); blood serum with swine (Kvasnickii, 1951); physiological saline or rabbit blood serum with rabbits (Warwick et al., 1943); aqueous humor from sheep's eyes with sheep (Warwick and Berry, 1949); physiological saline or Locke's with sheep (Casida et al., 1944); and homologous blood serum with cattle (Willett et al., 1953). Other media that have been tried for culturing eggs are described by Pincus (1936) or by workers cited by him. He found Ringer-Locke solution with an equal volume of homologous blood serum to be more satisfactory than balanced salt solutions. In later work Pincus (1939) tried Tyrode and other solutions and found them inferior to blood serum. Eggs kept in a balanced salt solution disintegrated after two hours. In general, media that have been found most satisfactory by most workers usually consist in whole or in part of blood serum.

Gates and Runner (1952) in a well-designed experiment with mice compared Locke's solution with Ortho bovine semen-diluter containing egg yolk and found the semen diluter to be superior as a medium for eggs prior to transplantation. Hammond (1949b) cultured mouse eggs in a medium including a number of salts and egg white and yolk.

Chang (1949) demonstrated that blood serum of man, sheep, cattle, goat, and fowl contain an ovicidal factor with the concentration or strength increasing among these species in the order given. He found no such factor in blood serum of rabbit, horse, dog, guinea pig, rat, and pig. Comparisons of sera were made by means of rabbit ova.

Chang (1947, 1948a, 1948b, 1950a, 1952) has also demonstrated that

fertilized rabbit eggs in various stages of development can be stored at refrigeration temperature for a day or longer and still remain viable. The optimum temperature was 10° C. A means of preserving eggs for still longer periods of time has been suggested by the freezing of rat eggs by Smith (1952). After being brought to a temperature of -79° C. in a medium containing glycerol and thawed, the eggs retained their normal appearance.

A large proportion of the work with media for mammalian eggs in vitro has been done with rabbits. The eggs of this species differ from most other species in that they become covered with a layer of albumin during their passage down the fallopian tube. Although no definite proof is available, this albuminous layer may afford protection to the cells during manipulation and storage in vitro. For this reason, the eggs of other species may be more easily harmed by such abnormal treatment, and the information derived from work with rabbits can not be applied to embryos of domestic animals without additional testing with the species concerned.

Solutions now developed may be fairly satisfactory for holding eggs for a few minutes or hours. When eggs are cultured, only a few cell divisions can be obtained with the media and techniques now available (Chang, 1947; 1948b; Hammond, 1949b; Nicholas, 1938; Pincus, 1936, 1939; Waterman, 1933, 1934). Lewis and Gregory (1929) observed rabbit eggs developing from early stages of cleavage to blastocysts. Nicholas (1938) found that embryos developed better in a circulating medium than in a still one. He also made some observations concerning effect of temperature, pH, and media upon development of embryos in vitro. The embryos he studied were more advanced in development than those suitable for transplantation. Pincus (1941) studied factors affecting development of rabbit blastocysts in vitro. The respiratory metabolism of mammalian eggs has been measured by Boell and Nicholas (1948) and Smith and Kleiber (1950).

INOVULATION

Beatty (1951) has suggested the word "inovulation" for the direct, non-operative transplantation of eggs because of its analogy with the word "insemination." It would appear logical to apply this term to any method, surgical or non-surgical, of placing an egg in the female.

It has been demonstrated by Nicholas (1933) with rats, Fekete and Little (1942) with mice, and Chang (1950b) with rabbits that the stage of the estrual cycle of donor and recipient must be closely synchronized. In addition, Whitney and Burdick (1938) found that eggs accelerated in their passage down the fallopian tubes degenerated upon reaching the uterus. The uterus has to undergo progestational proliferation before it is capable of promoting embryonic development.

Synchronization of the estrual cycles of donor and recipient may be facilitated by altering the estrual cycle of the recipient with progesterone (Ulberg et al., 1951). Ewes treated in this way were normal in fertility (Casida et al., 1945; O'Mary et al., 1950). Willett (1950) obtained a fairly satisfactory conception rate in heifers inseminated at the first heat following administration of this hormone. Asdell (1952), however, reported low breeding efficiency in heifers following such treatment.

Estrus following such treatment may, therefore, not always be normal. The cycle of the recipient in one of the successful transplantations by Willett et al. (1953) had been altered with progesterone. Limited experience has indicated that control with progesterone of the time of estrus of the recipient, and not of the superovulated donor, is adequate.

With two exceptions, all reports of successful inovulations in mammals have involved surgery:

Rabbit (Avis and Sawin, 1951; Black et al., 1951; Biedl et al., 1922; Chang, 1947, 1948a, 1948b, 1949, 1950a, 1950b, 1952; Dowling, 1949; Heape, 1890, 1897; Pincus, 1936, 1939; Serebrjakov and Kraševeninnikova, 1951; Venge, 1950).

Rats (Nicholas, 1933; Noyes, 1952).

Mice (Fekete, 1947; Fekete and Little, 1942; Gates and Runner, 1952; Runner, 1951).

Cows (Willett et al., 1953).

Goats (Warwick and Berry, 1949).

Sheep (Casida et al., 1944; Lopyrin et al., 1950, 1951; Warwick and Berry, 1949).

Swine (Kvasnickiĭ, 1951).

The two exceptions were Kvasnickiĭ (1951) who reports one pregnancy in a sow resulting from eggs placed in the uterus via the vagina, and Beatty (1951) who transplanted 99 eggs into the uteri of mice by the same approach. Five transferred embryos were recovered as young born. Umbaugh (1949), Dowling (1949), and Rowson (1951) have placed bovine eggs in the recipient's uterus by way of the vagina and cervix without success.

These unsatisfactory results when transferring ova into the female through the vagina and cervix have been shown to be due to the susceptibility of the uterus to infection during the luteal phase of the estrual cycle and to the development of pyometra. Willett et al. (1948, 1952a), Rowson (1951), and Brock and Rowson (1952) in the cow; Tanabe et al. (1949) in swine; and Murphree et al. (1951), Black et al. (1951), McDonald et al. (1952) in rabbits have observed this condition when semen was placed in the uterus when an active corpus luteum was present. Rowson et al. (1953a) and Black et al (1953) have shown that the uterus is highly susceptible to infection during the luteal phase and highly resistant during the follicular phase. Lamming and Rowson (1952) and Brock and Rowson (1952) have concluded that the development of pyometra precludes normal development and implantation of eggs inovulated by way of the vagina and cervix. They found it impossible to avoid some infection. In further studies Rowson et al. (1953) found that antibacterial agents in the diluted semen eliminated or reduced the severity of the metritis. These results with antibiotics suggest that ways may be found to avoid infection when transplanting eggs into the uterus per vaginam.

Lamming and Rowson (1952) have attempted to overcome this difficulty by embedding tubal eggs in gelatin in a small cup at one end of a small glass dart and by means of a long tube and plunger inserting the dart into the ovarian ligament close to the ovary. This was done by passing the tube through the vagina and cervix, into the uterus, through the uterine wall and into the abdominal cavity, and then plunging the dart into the ovarian ligament. Barbs on the dart retained it in position. The gelatin

melted and liberated the egg. A non-surgical operation of this nature would be done shortly after estrus and can be done very quickly. The disadvantage of this procedure is that tubal eggs must be used. If egg transplantation is to be made a fairly simple and practical technique comparable to artificial insemination, extraction of eggs from the uterus without surgery appears to be the only means possible. Uterine eggs must be placed in the uterus and not in the tubes as would be done with Lamming and Rowson's dart.

This dart or some comparable procedure may be practical, however, under the conditions existing in England. Almost all slaughtering of meat animals is done in government-operated abattoirs. Hammond (1950) anticipated the possibility of superovulating beef cows soon to be slaughtered, extracting fertilized tubal eggs after killing, and placing them in dairy cows. In this way beef calves could be produced by dairy cows.

With inovulation by means of laparotomy, the recipient must, of course, be anesthetized. Kile (1951), and Willett et al. (1953) have raised the question of the possible effect of anesthesia upon fertility and embryonic mortality, respectively. Willett et al. suggested that complete anesthetization with chloral hydrate, Nembutal, and ether may have been one of several possible factors responsible for their unsuccessful transplantations. More recent experiments (Buckner and Willett, 1953) with mice suggested that either Nembutal, chloral hydrate, or ether administered early in pregnancy causes loss of whole litters during pregnancy . The results were, however, inconclusive.

When transplanting eggs one should place in the recipient approximately the number normally ovulated. Evans and Simpson (1940) with rats, Casida et al (1944) with sheep, Hammond (1949a) with cows, and other workers found that, although ovulations were increased by exogenous gonadotrophin, the number of young born or potential young were little greater and sometimes less than the normal number. Hammond found that cows with more than three corpora and, therefore, with apparently more than three fetuses aborted before five months of gestation.

Data obtained by several workers may suffice to illustrate the percentage survival of transplanted eggs with present methods. Fekete (1947) transplanted 5,046 mouse eggs, and 11.4 to 18.2 per cent developed to young born. Corresponding figures for others were: Venge (1950), 1,478 rabbit eggs, 28 per cent; Chang (1950b), 532 rabbit eggs, 29 to 62 per cent; Nicholas (1933), 38 rat eggs, 60 to 80 per cent; Warwick et al. (1949), 27 sheep and goat intra-specific transplants, 22 per cent.

DISCUSSION

Various benefits that might be derived from egg transplantation have been postulated:

(a) The rate of progress in genetic improvement of livestock would be increased. Comstock (1949) has estimated this increase as compared with present methods of breeding. Progress with dairy cattle was estimated to be 1.5 to 2.0 times that in which selection of females is intra-herd on basis of their own performance and selection of males was inter-herd on the basis of full-sister performance. In actual practice the increase would not be this much because maximum intensity of selection

was assumed. On the other hand, transplantation might make it possible to use as dams only females selected on basis of incidence of mastitis or other characteristics that can not be effectively selected for now. Progress expected with transplantation in beef cattle was estimated to be greater than with dairy cattle because beef cattle have traits measurable in the male as well as the female. With 50 progeny raised per sow per year, progress with transplantation would be 1.79 to 2.10 times greater than with conventional methods.

A similar study was made by Kyle (1949). He considered egg transplantation as being of little advantage with swine. With dairy cattle, beef cattle, and sheep, in the first generation the estimated maximum rate of progress with transplantation was 2.5 to 3.5 times present rates. These studies by Comstock and Kyle assumed that eggs could be recovered repeatedly and regularly from donors which have been superovulated. Both estimated that rate of progress would be considerably reduced after the first generation.

(b) Cows could be effectively progeny tested at a fairly early age.

(c) The development of inbred lines would be facilitated.

(d) If numbers of fertilized eggs could be procured from calves and transplanted to sexually mature recipients, the generation time of cattle could be reduced to one year. Several successive generations could be sired by one male. Black et al. (1953b) have studied the possibility of superovulating calves. The ovulation rates were low. Marden (1952), however, obtained satisfactory ovulation rates by injecting FSH three weeks prior to superovulation. An "experimental corpus luteum" was formed. In both these studies only a small percentage of ovulated eggs had cleaved although most of the calves were inseminated.

(e) Fertilized eggs could be transported long distances. Marden and Chang (1952) obtained young from recipients in England with donors in the United States. The embryos were shipped by air.

(f) Egg transplantation would make possible more fundamental approaches to the problems of animal breeding, genetics, and the physiology of reproduction. To cite a few examples, reciprocal transplantations of eggs between normal and hard-to-settle cows would aid in determining whether the egg or the uterus is at fault in partial or complete physiological sterility. The male, as well as the female, may also be responsible for abnormal embryos, for Bouricius (1948) found a large number of abnormal appearing 4- to 8-cell embryos in normal female rats mated to partially sterile males. Nicholas and Hall (1934, 1942) transplanted isolated blastomeres in rats and observed initial development, mucosal reactions, decidua formation, and egg cylinders. All embryos were finally resorbed. Additional research along this line might enable production of many monozygous individuals. Briggs and King (1952) have had frogs develop from eggs from which nuclei were removed and replaced with those from blastula cells. Beatty and Fischberg (1949) have induced polyploidy in mouse eggs. Russell (1948), Fekete and Little (1942), and Venge (1950) have published data which suggested that uterine environment influences, respectively, number of vertebrae, incidence of carcinomas in mice, and body size. Economic traits of farm animals may also be influenced by uterine environment.

When one considers the present state of development of superovulation

and egg transplantation as a tool for the genetic improvement of dairy cattle, it can be seen that considerable progress has been made but that much more work is needed. Little has been done with other farm animals. Fertilized bovine eggs in numbers can be produced by superovulation. Several workers have extracted eggs from cows without surgery. Calves, sheep, and pigs have developed from transplanted eggs.

Present data indicate, however, that an individual cow can be superovulated only a few times. Additional research may show how to overcome this difficulty. The recovery of eggs from cows without surgery has been inefficient. More data are needed to establish definitely whether fertilized superovulated eggs are as capable of normal development on the whole as fertilized normally ovulated eggs. Little is known about media, pH, and other facts necessary for optimum conditions for eggs in vitro, either for storage or for culture. A completely satisfactory method of inovulation has yet to be demonstrated. Due to susceptibility of the uterus to infection when an active corpus luteum is present, Lamming and Rowson (1952) expressed the opinion that overcoming this problem may be the key to successful methods of transplantation not involving surgery in cattle.

Will superovulation and egg transplantation ever be a practical tool for the animal breeder? It was originally thought that eventually technicians would be carrying fertilized eggs from farm to farm like diluted semen is now. Spermatozoa can be collected from the bulls by the billions; the theoretical possibilities of artificially inseminating cows are unlimited. The total number of eggs that can be procured from an individual cow now appears to be a few dozen. Unless ways are found to procure eggs in large numbers, or to compensate for the limited number of eggs by finding ways of successfully obtaining and transplanting individual blastomeres, the improvement of the population by mass application of superovulation and egg transplantation is out of the question.

The techniques might find most practical application in large breeding establishments and government experiment stations or in countries where abattoirs are nationalized as in England or where farms are collectivized as in Russia. Large breeding establishments in this country have already considered the possibility of superovulating aged outstanding cows, killing them, and transplanting their eggs. Some establishments would be interested in doing this now if they could be assured of some degree of success. Even if surgery were necessary for inovulation, they could afford the expense.

The greatest benefits probably would be derived from the technique as a tool for research workers in physiology of reproduction and in genetics. Here surgery would not necessarily be a limiting factor. A by-product of research in this field is expansion of knowledge of reproduction. These possibilities alone justify continued research and development.

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THE FUTURE OF OVA TRANSFER

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Ova transfer or inovulation includes the recovery of ova from a donor and their subsequent transference to a suitable recipient. The development of this procedure is important to fundamental investigations in the field of physiology of reproduction, and has applications in the field of animal husbandry. For example, it would be possible to recover ova at oestrus from outstanding cows and transfer them to less desirable individuals for subsequent embryological and foetal development. Through such a procedure it would be possible to make use of the germ plasm available from superior females in the same way that artificial insemination has increased the usefulness of superior sires.

This discussion is not directed toward analyzing all the possibilities of ova transfer, but will deal with some of the current problems confronting investigators in this field. In order for ova transfer to become a practical reality, the following factors must be taken into consideration:

1. Economic aspects of the technique.
2. Simplicity of procedures involved.
3. Assurance that the proper offspring is born.

To achieve the preceding objectives, many fundamentals must be studied. Among these are:

1. Superovulation or the production of more than one ovum per oestrus.
2. Synchronization of the oestrus periods of the cows.
3. Simple, non-surgical methods for recovering ova.
4. Media suitable for maintaining ova over long periods of time.
5. Simple methods for introduction of ova into recipient cows.

It has been found that superovulation may be induced upon the administration of PMS, whereupon a refractiveness begins to appear. Willett et al. (1952) have shown that there is a decreasing number of ova liberated with subsequent attempts to produce superovulation with PMS. The exact cause of this decrease in responsiveness is unknown. It is necessary to stress that our present knowledge of the mechanism of hormone action as well as hormonal interrelationships is incomplete. PMS may cause an alteration in hormone equilibrium in the cow which is not easily overcome. It is possible that superovulation in the bovine may be controlled if the required amounts of FSH, estrogen, LH, and progesterone could be supplied at the proper time of the ovarian cycle. The cow ovulates one ovum or possibly a few ova at each oestrus. Superovulation causes the rapid production of quickly matured ova, and as a result a considerable period of time must elapse before others are sufficiently

developed for liberation. This delay in maturation may contribute to the observed refractiveness. Unpublished data of the author suggest that cows become refractory toward the administration of PMS after the first oestrus. However, this refractiveness apparently is not due to the production of anti-hormones or antagonistic substances as assayed on young rats. Inasmuch as PMS or any of the follicle-stimulating hormone preparations used are not homogeneous, there is always a possibility that some impurity may be the cause of the existing refractiveness.

Another major problem of inovulation, while in the experimental stages, is that of synchronizing the oestrus periods; this phase will be of minor significance when a sufficient number of ova can be stored for some time. With a limited number of animals, the recipient must be in the same state of oestrus as the donor, but with large numbers of recipients some will always be synchronized with the donor. Although the author has used follicle-stimulating hormone and luteinizing hormone in attempts to synchronize the oestrus cycle, all trials have failed. The use of progesterone, at present, seems to be the most promising technique. No difficulty has been experienced in synchronizing the oestrus periods of cows when 50 mg. of progesterone were injected subcutaneously daily, starting on the fourteenth day of the oestrus period and continuing as long as desired. At the cessation of injections, the cows were in heat on the fourth day plus or minus 12 hours. At the present time, this 12 hour interval does not seem to be detrimental to ova transfer. Therefore, apparently the problem of synchronizing bovine oestrus periods can be controlled.

While superovulation and synchronization of the oestrus periods are important, probably the most perplexing undertaking of the entire field of inovulation is the recovery of viable ova. Although the transplantation of fertilized ova is not new, its application to farm animals is far from being perfected. Dracy and Peterson (1950) performed many exploratory experiments in attempts to recover ova. These early attempts included removal of the ovary from its original site and transplanting it into the neck of the animal so that it was easily accessible. However, none of the transplants was successful. Later they attempted to transfer the ovary from its site deep in the pelvic arch to a position under the skin. This, however, again resulted in failures probably due to reduced environmental temperature. Another approach was to resect one horn of the uterus, connecting to the exterior, thus allowing the ova to be recovered. Due to the lack of antibiotics at the time of the experiment and possibly due to other unknown factors, including surgical techniques, metritis and salpingitis resulted. Therefore, these experiments were failures. Next, the ovary was translocated into the vagina, the object being to have the ovary in a position to be observed with a speculum and so placed that the ovum could be recovered at the time of its liberation. The procedure was unsuccessful, inasmuch as traction applied by the broad ligament was great enough to pull the elastic wall of the vagina far enough forward to encase the ovary, thus rendering it non-functional.

A more satisfactory surgical method has been developed by Umbaugh (1949) whereby laparotomy exposes the ovary, fallopian tube, and uterine horn. In this technique, a catheter was inserted into the uterine horn against the end of the fallopian tube, and blood serum was used as the

medium for forcing the ovum out of the fallopian tube two or three days after the cow had ovulated. Of all the surgical methods thus far developed, this is probably the most successful, but it has the following limitations:

1. A large incision must be made. This, of course, increases the possibility of infection. The number of incisions that can be made on the side of any one cow is limited.

2. After the uterine horn is opened in order to insert the catheter, there is the possibility that the walls of the horn may grow together at this point, thus closing the fallopian tube and rendering it impossible for the ova to travel down, or for fluid to be pushed back after the first recovery.

3. Technical skill is required in the surgical procedure.

Since surgical procedures seem to be undesirable, attention has been directed toward non-surgical means of flushing the uterus to obtain the ova. Dracy and Petersen (1950) have devised a method whereby the uterus can be flushed with rather large quantities of physiological saline and the ova recovered. Recovery of ova succeeded in 12 out of 37 experiments in which this procedure was used. This method of recovering ova has several limitations. Among these is the fact that a large quantity of liquid must be used to recover a microscopic ovum.

Since modifications are necessary, Rowson and Dowling (1949) have devised a three-way catheter that apparently is superior to the above flushing method in that it allows the operator to use a relatively small quantity of fluid. A procedure which would relax the cervix would be useful in the collection of ova inasmuch as the cervix is constricted at the time of the collection. In this connection, Graham (unpublished M.S. thesis, South Dakota State College) injected varying quantities of relaxin to obtain cervical relaxation. In this series of experiments, he was able to obtain relaxation up to 1.61 inches by the subcutaneous injection of 1500 guinea pig units of relaxin. The cervical dilation might allow entrance into the uterus so that the ovum could be recovered with reduced quantities of fluid or possibly no fluid at all. The possibility of recovering the ovum would be enhanced if such a technique could be devised. There is the possibility of inducing infection any time the cervix is dilated and the uterus entered. However, Wulf (unpublished M. S. thesis, South Dakota State College) has conducted a number of experiments to determine whether or not metritis resulted from flushing the cervix with sterile solutions. In some of these experiments bacteria were recovered, while in others none were found. However, infection did not result from the use of sterile instruments and sterile fluids within the uterus. Thus, it would seem that if proper techniques are applied, the uterus can be irrigated period after period without detrimental results.

The ova should be recovered when they are in the fallopian tube, for then only a small amount of fluid is necessary for flushing. However, if the ova are to be recovered from the uterus, another problem is at hand. Enough time must elapse for the ova to travel from the ovary to the horn of the uterus. In general, this is reasonably well established as 4 days (Winters et al., 1942). Thus, when the irrigation procedure is used, the ova must be at least 4 days old, which makes them considerably older than when they are recovered directly from the fallopian tube. That the

recovery of ova after 4 or more days is detrimental to their survival remains to be seen. The zona pellucida becomes thinner with the ageing of the ovum while the vitelline membrane increases. As a result of these changes, it is possible to assume that the older ova may disintegrate more quickly under slightly abnormal conditions. It is suggested that the flushing should be done prior to the seventh day, possibly on the fifth day, inasmuch as Dracy et al., (1950) recovered ova on the seventh day post oestrus.

As has been pointed out by a number of investigators, the most desirable medium for recovering the ova is blood serum. In recovering ova from a fallopian tube, small quantities of blood serum are easily handled. However, if large quantities of fluid are necessary for flushing the uterus, blood serum becomes less desirable because of a lack of transparency. In this connection, many of the experiments have been conducted using physiological saline. According to Chang (1949), these salt solutions are undesirable for ova collection and this may be the reason that previous experiments frequently failed to produce ova.

Another problem, in connection with flushing the uterus, is that in some trials small quantities of blood are present in the uterus due to trauma. In the experiments conducted by the author, very little injury occurred; therefore, a clear solution was usually recovered. It would be difficult to recover the ova from fluid containing blood, because for any solution to be desirable for flushing the uterus, it must be nearly transparent. Therefore, a desirable solution for this purpose would have the same physiological properties as blood serum and yet be transparent.

After the ova have been recovered from the donor, some means of preservation should be developed whereby they can be maintained in a viable condition for an extended period of time. Chang (1947) has been able to preserve rabbit ova at a low temperature for several days and then transplant them into recipients and produce living young. Whether or not this is entirely applicable to cattle remains to be seen. There is the possibility that ova might be stored in a frozen condition. This seems possible since under optimum conditions sperm can be frozen to -79° C. and held for a prolonged period of time (Miller and VanDemark, 1953). Therefore, with the proper medium and conditions, it seems logical to assume that ova could be kept in a viable condition for a period of time.

Another important aspect of the problem remains to be considered. To date, Willett et al. (1953) have produced three living calves by the transplantation of fertilized ova. All of these transplants have been accomplished by surgery. However, if transplantation is to be economic, surgical procedures are not desirable. The introduction of ova by means of gelatin capsules has resulted in pregnancy in two cows at South Dakota State College. However, fetal development did not continue beyond three months; whether this was due to infection or injury to the recipient is not known.

Ova transfer also produces the problem of the proper identification of the offspring from the mating. There will be the problem of getting the breed associations to accept this new means of securing offspring.

In conclusion, it may be said that superovulation will become more useful when some of the present difficulties have been overcome, particularly those involving hormonal balance. The synchronization of the

oestrus cycle in the recipient cow can be controlled by the subcutaneous injection of 50 mg. of progesterone daily after the fourteenth day post oestrus for any period of time. Ovulation occurs 4 days following cessation of hormone injection. Surgical procedures are successful in the recovery of ova from donors following superovulation. However, the method of flushing them from the uterus is more advantageous than the surgical methods available at this time.

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MOTILITY PATTERNS IN THE FEMALE REPRODUCTIVE TRACT

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It has long been known that the reproductive tract of the female exhibits patterns of motility which vary with the other changes that occur in the reproductive cycle. These changes have been described in some species during both the estrous cycle and pregnancy. Since the predominating hormonal influence changes during the estrous cycle, the motility pattern exhibited at any one particular time generally has been attributed to the hormone dominant at that time. Functions that have been ascribed to the activity of the reproductive tract have included sperm transport in the female tract, ovum transportation, and phenomena involved in pregnancy and the expulsion of the fetus. It is not intended to consider here the implications of activity of the reproductive tract during pregnancy or parturition.

While numerous studies have been made concerning the motility of the female reproductive tract of laboratory animals, investigations involving the larger farm animals have been limited. In the reports pertaining to both laboratory animals and larger animals, differences have appeared and some of these seem to have plausible explanations, while others do not. Some of these differences, as they pertain to farm animals particularly, are discussed in the following review.

SPONTANEOUS MOTILITY

Reynolds (1949) has made a comprehensive review of the spontaneous motility in the female reproductive tract of laboratory animals. He points out that, in general, under the influence of estrogens, activity is increased and under the effects of progesterone activity decreases. Many of the studies to which he refers were done in vitro using uterine strips in oxygenated baths of Locke's or other similar solution.

Evidence on the motility of the female reproductive tract of the larger farm animals is limited. The reviewers have found reports of the motility patterns in the sow, ewe, and cow.

Sow. Studies on the spontaneous activity of the sow uterus have been made by Keye (1923). He found that transverse strips showed two types of contractions. These consisted of relatively large contractions of long duration (1.5 to 2.5 minutes) and smaller contractions superimposed on the larger ones. The large contractions persisted during the maturation of the follicles and for a short time after ovulation. When the corpora lutea reached maturity (about the 7th day after ovulation) and until regression began, the smaller contractions were predominate or were present alone.

The report on uterine activity in the sow referred to above is contrary to the findings reported by Seckinger (1923) on the motility patterns of the fallopian tubes of the sow. Using segments of the oviducts in in vitro studies, Seckinger found that small, rapid contractions began as estrus approached and persisted during estrus. These rapid contractions (13 to 15 per minute) terminated after the follicles had ruptured and the ova had entered the uterus (4th day). From the 4th to about the 19th day slow (4 to 6 per minute) undulating contractions of equal amplitude (much larger than the type found during estrus) predominated. Tubal contractions during early pregnancy were similar to those observed at the interestrous period.

Wislocki and Guttmacher (1924) studied the spontaneous activity of the reproductive tract of the sow by placing the whole excised tract into a warm tank of oxygenated Locke's solution. They observed that the activity of the uterus and the tubes began to increase on approximately the 19th day of the estrous cycle along with the rapid growth of follicles. By the 21st day, the contractions had increased from moderate to vigorous or very vigorous. From the first to the third day, very vigorous tubal contractions continued but uterine activity subsided a little. Diminution in the activity of both continued until both were called moderate at the end of the 3rd to 7th day period. From the 7th to the 15th day, activity diminished from moderate to feeble or very feeble, and contractions during the 15th to 19th days were very feeble or completely absent.

The observations of King (1927) served to clarify some of the results obtained by Keye and Wislocki and Guttmacher. King recorded the contractions of both longitudinal and circular strips of the sow uterus. The results obtained indicated that the action in the unseparated segments was considerably diminished compared to that shown when the separate portions were used. Apparently, the action of each is inhibited by the presence of the other. King reported that the activity of both layers was at a maximum during estrus and at a minimum from the 4th to the 16th day of the cycle. Yet, an examination of the recordings presented by King does not clearly reveal the changes claimed. The contractions of the longitudinal strips appear to be of approximately the same amplitude in some of the estrous and postestrous recordings. However, it was reported that the working power (the ability to raise weight during contractions) of the longitudinal muscle strips paralleled its activity during the estrous cycle, and the figures given indicate a reduced working power during the 4th to 16th day of the cycle.

Ewe. The spontaneous activity of the reproductive tract of the ewe was noted by Ambache and Hammond (1949). They observed fast contractions of 2-3 per minute in uterine strips from the anestrous ewe. A similar rhythm but weaker and less regular was observed in the uterus of a pregnant and in that of a non-pregnant sheep with a corpus luteum present in the ovary. Most strips from pregnant ewes showed no spontaneous rhythmic activity.

Cow. In in vitro studies using uterine strips from the cow, Cupps and Asdell (1944) found that spontaneous motility varied with the estrous cycle. During proestrus, estrus, and metestrus (until about 2 days postestrus) the motility pattern of longitudinal strips consisted of slow even contractions of considerable magnitude recurring at 1.5 to 2.0 minute

intervals. A tendency toward tonic contraction for a few minutes was evident during estrus and the day after. Superimposed over slow contractions were small rapid contractions at 20 to 30 second intervals. After 2 days postestrus they reported that the activity gradually decreased with the small rapid contractions becoming more pronounced and with long regular changes in tonus becoming evident giving a general pattern of irregular activity. By eight days postestrus they found little spontaneous activity and at 12 to 16 days postestrus activity was even less. At two days proestrus activity began to reappear in the form of irregular contractions.

Evans and Miller (1936) made recordings of uterine activity in the cow using the balloon technique in vivo. Their observations on spontaneous uterine activity were similar to those noted later by Cupps and Asdell in vitro. These observations consisted of marked contractions during estrus and for a few days postestrus, then the contractions became slight during the diestrous period.

Hays and VanDemark (1953a) studied the spontaneous activity in the bovine uterus at various stages of the estrous cycle using the balloon technique in the intact cow. They found that the amplitude and frequency of uterine contractions varied during the estrous cycle. However, they pointed out that although there were changes in the activity it was important to define the changes according to amplitude and frequency, for the product of these did not change significantly during the cycle for any one cow. During estrus and the day following, the spontaneous contractions were regular, frequent (4-4.5/min.), and of small amplitude. By the 4th to 5th day of the cycle, the frequency of the contractions had diminished, the amplitude had changed so that a few small contractions were superimposed on larger contractions, and irregular changes in the tone had begun to appear. These changes became more pronounced by the 11th to 12th day of the cycle with the frequency decreasing to 2.5-3 contractions per minute. At 1 to 2 days proestrus, the irregular changes in tone disappeared but the amplitude and frequency of the contractions did not change greatly from that observed at mid-cycle. Representative tracings of uterine activity at estrus and at mid-cycle are shown in Fig. 1(1) and 1(2), respectively. The maximal contractions observed by these investigators represented changes in pressure of approximately 30 to 40 mm. of mercury.

The activity indices presented by Hays and VanDemark (1953a) are shown in Table 1. An analysis of variance indicated that there were no significant differences in the motility indices for various stages of the estrous cycle. Significant differences were observed between cows. Following the observations at various stages of the estrous cycle, cow number 4 was ovariectomized. Between 90 and 265 days after ovariectomy the uterine activity indices were: with no treatment, 3.8; after stilbesterol, 7.0; after progesterone, 7.4; and after stilbesterol followed by progesterone, 6.1. This was in agreement with the findings of many workers that ovariectomy reduced uterine activity, but the authors were surprised to find that progesterone alone, as well as estrogen, returned the activity (as measured by the activity index) to normal. The motility patterns in the uterus of the ovariectomized cow under the influence of stilbesterol and progesterone corresponded well with the patterns shown in the intact cow under the influence of these hormones during the estrous cycle.

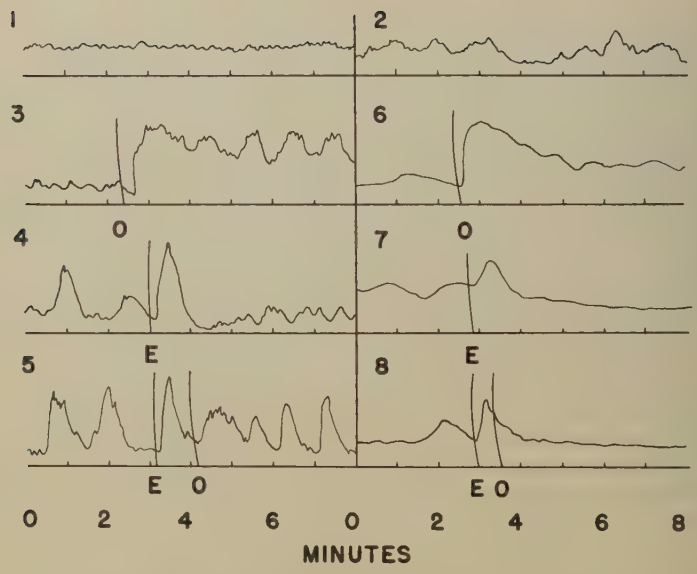

Fig. 1. Kymograph records of motility in the bovine uterus. (1) Spontaneous motility during estrus, (2) spontaneous motility during mid-cycle, (3-5) in vivo, and (6-8) in vitro responses to oxytocin (O) and epinephrine (E) injections or additions.

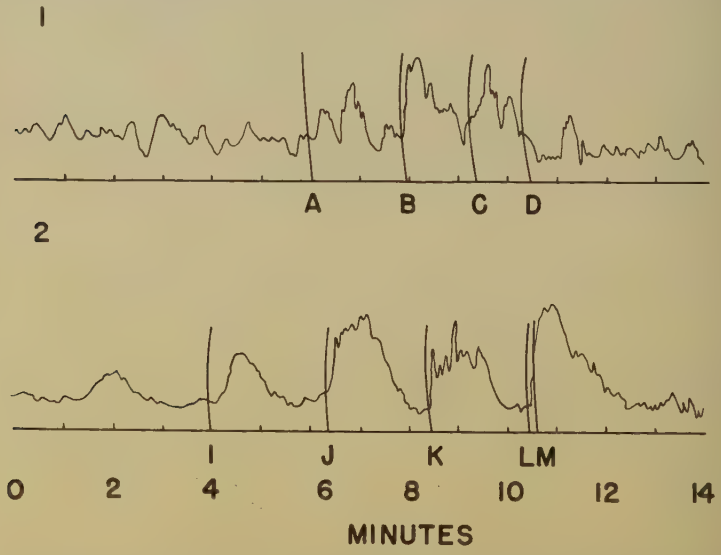

Fig. 2. Uterine responses to artificial insemination (1) and natural mating (2). A-B, massage of the anal region; B-C massage of the cervix; C-D insertion of the inseminating tube; I, bull brought into the sight of the cow; J, bull allowed to nuzzle the vulva and rear quarters of the cow; K, bull allowed to mount but not copulate; L, bull allowed to mount and copulate; M, bull ejaculated.

Two opposite points of view concerning the spontaneous activity of the reproductive tract of farm animals seem to exist from the findings reviewed. One, based on in vitro studies with the sow and both in vivo and in vitro studies with the cow, holds that activity is greatest at estrus and gradually diminishes to little or no activity a few days after estrus. Little activity then is shown until a short time before the next estrus. The other point of view is based on the findings that (in the tubes of the sow and the uterus of the cow) small sharp contractions occur at a rather high frequency at and following estrus and these gradually give way to contractions of greater amplitude but of a slower frequency. This type of contraction then persists until near the onset of the next estrus.

TABLE I

Activity Indices (Frequency of Contraction/Minute Times Average Amplitude in mm.) of Bovine Uteri During the Estrous Cycle

Cow No.	Days Postestrus 0-1	3-4	10-11	Days Proestrus 1-2
1	12.1	8.8	9.1	8.2
2	7.1	6.1	5.7	7.1
3	7.1	6.4	7.6	6.4
4	6.8	7.6	8.1	7.5

From Hays and VanDemark (1953a).

From the evidence presented in the literature it is uncertain whether a plausible explanation for the different findings can be made. As was indicated by King (1927), the longitudinal muscles of the reproductive organs of the sow have a different contraction pattern than do the circular muscles. If this holds true for all animals, then the findings of those who used the whole tract might have been expected to differ from the findings where only segments were used.

Another possible cause for the varying reports is probably due to the failure to carefully distinguish and define the amplitude and frequency of the contractions observed. If recordings were not made of the contraction pattern, slow contractions resulting in considerable change might easily go unobserved while rapidly recurring small contractions would be noticed. The findings reported by Hays and VanDemark (1953a) indicated that the amplitude and frequency vary inversely in the uterus of the cow. The motility pattern recorded by these investigators for the uterus of the cow was similar to the pattern recorded by Seckinger (1923) for the tubes of the sow at comparable stages of the estrous cycle. Both of these indicate that rapid small contractions take place under the influence of estrogen and that these give way to larger slower recurring contractions during the luteal phase of the cycle.

HORMONAL EFFECTS

The effects of the hormones from the posterior pituitary and from the adrenal medulla on the motility of the reproductive organs have attracted much attention. As a result, there appear in the literature numerous reports dealing with the effects of oxytocin and epinephrine on uterine motility especially in laboratory animals. Many of these reports have been reviewed by Reynolds (1949). In most small animals oxytocin causes uterine contractions at the various phases of the estrous cycle and during pregnancy. However, in some, progesterone decreases the reactivity to oxytocin. As a result of the epinephrine effects on the reproductive tract, especially in small animals, three classes of animals have been defined. Group 1, the rabbit group, is characterized by uterine contractions to epinephrine both in the presence and absence of progesterone. In group 2, the guinea pig group, epinephrine is an inhibitor both to the nongravid and gravid uterus. In group 3, the cat group, epinephrine is inhibitory in the absence of progesterone and motor in its presence. However, within these groups one finds that exceptions have been reported.

The effects of oxytocin and epinephrine on the motility of the female reproductive organs of farm animals have not been studied extensively. Even in the limited number of reports which have appeared, differences in responses have been observed.

Ewe. Alexander (1945) and Ambache and Hammond (1949) found that uterine strips from both pregnant and non-pregnant ewes responded to oxytocin by strong tetanic contractions. Alexander found that epinephrine (adrenaline) brought about an inhibition of uterine activity in both the non-pregnant and the pregnant ewe. Earlier, Gunn (1944) had reported that epinephrine (adrenaline), at concentrations similar to those used by Alexander, produced a motor effect on the non-pregnant sheep uterus and an inhibition of activity and a reduction of tone in uterine strips from the pregnant ewe. Neither of these two investigators indicated whether or not the animals used were in anestrus. Ambache and Hammond (1949) used uterine strips from anestrous ewes and found a motor effect of adrenaline. In some instances, during pregnancy the response to adrenaline was a brief motor effect followed by relaxation.

Cow. Cupps and Asdell (1944) found that uterine strips contracted and remained in tonic contraction under the influence of pituitrin at all stages of the estrous cycle. Alexander (1945) also found that uterine strips of both the non-pregnant and pregnant cow underwent strong tonic contractions when posterior pituitary extract was added to the bath.

Contrary to the above-mentioned observations, Evans and Miller (1936) reported earlier that the uterus of the intact cow became refractory to pituitrin after ovulation. However, more recent studies by VanDemark and Hays (1951) and Hays and VanDemark (1953b) are not in agreement with the latter report, but agree with the in vitro studies mentioned first. In in vitro studies these investigators found that the excised perfused reproductive tract of the cow responded by tetanic contractions when oxytocin (pitocin) was added to the perfusate. In the intact animal intravenous oxytocin injections caused tetanic contractions to occur in the uterus within a few seconds. An example of the response of the intact uterus to oxytocin is shown in Fig. 1(3); an in vitro response is

shown in Fig. 1(6). No detectable differences in response were noted at various stages of the estrous cycle. However, in an ovariectomized animal, the uterine response to oxytocin was greatly diminished but was returned to the near normal response when the animal was treated with diethylstilbesterol. In the ovariectomized animal under the influence of progesterone, the response of the uterus to oxytocin was greatly reduced.

The effects of epinephrine on the uterus of the cow have been somewhat controversial. Cupps and Asdell (1944) reported that the response to epinephrine varied with the stage of the cycle. They indicated that from estrus to four days postestrus, as a rule, epinephrine caused an inhibition of uterine muscle contractions while during diestrus a motor effect was frequently observed. Alexander (1945), on the other hand, found epinephrine (adrenaline) caused a diphasic effect on uterine strips. His recordings show that with strips of both the non-pregnant and pregnant cow, the first response is one of contraction followed by a drop in uterine tone well below the pre-addition level. The results obtained by VanDemark and Hays (1951) and Hays and VanDemark (1953b) agree with the findings of Alexander (1945). In both in vitro and in vivo studies these investigators observed in a majority of the cases a diphasic response. Examples of the epinephrine effect on the bovine uterus in vivo and in vitro are shown in Fig. 1(4) and 1(7), respectively. While a reduction in uterine tone and diminished activity always was observed in vivo, occasionally there was no prior contraction wave. In an ovariectomized cow, the uterine response to epinephrine was slight, with little contraction and little reduction in tone being shown. Both of these responses to epinephrine were increased following stilbesterol and progesterone treatment. There was some indication that epinephrine caused a greater reduction in tone and activity after progesterone treatment.

Both in vitro and in vivo studies have shown that epinephrine effects on the bovine uterus will prevent the usual contractions caused by oxytocin. The diphasic response to the epinephrine usually occurs, but the oxytocin stimulation does not appear [see Fig. 1(5) and 1(8)].

The effects of oxytocin and epinephrine on the uteri of the ewe and the cow as they have appeared in the literature are summarized in Table 2. With the exception of the report by Evans and Miller (1936) that oxytocin produced no effect on the cow uterus during the diestrous period, all the investigators have observed that oxytocin produced a motor response throughout the estrous cycle and during pregnancy in both the cow and the ewe.

The reports on the effects of epinephrine have indicated more variation in the responses. In the ewe, both motor responses and inhibitory effects have been observed in the non-pregnant animal. In the pregnant ewe, it is agreed that epinephrine causes an inhibition of activity. Ambache and Hammond (1949) noted also that a diphasic response occurred with strips of the uterus from a pregnant ewe. Such responses were characterized by an initial contraction followed by a period of reduced activity. In the cow, with one exception, a diphasic response has been reported as the result of epinephrine treatment. Cupps and Asdell (1949) found that inhibition occurred in strips taken from the estrous cow and contractions resulted in those from the diestrous cow. Hays and VanDemark (1953b) found that the initial motor effect did not always occur.

TABLE 2

The Effect of Oxytocin and Epinephrine on the Activity of the Uterus of the Cow and the Ewe

Species	Oxytocin			Epinephrine			Reference
	Estrus	Diestrus	Preg.	Estrus	Diestrus	Preg.	
Cow							
In vitro	Motor	Motor	- -	Inhibit.	Motor	- -	Cupps and Asdell (1944)
In vitro	Motor		Motor	Diphasic (M-I)		Diphasic (M-I)	Alexander (1945)
In vitro	Motor	Motor	- -	Diphasic (M-I)*		- -	Hays and VanDemark (1952, 1953b)
In vivo	Motor	No effect	- -	- -	- -	- -	Evans and Miller (1936)
In vivo	Motor	Motor	- -	Diphasic (M-I)*		- -	VanDemark and Hays (1951) Hays and VanDemark (1953b)
Ewe							
In vitro	- -	- -	- -	Motor		Inhibit.	Gunn (1944)
In vitro	Motor		Motor	Inhibit.		Inhibit.	Alexander (1945)
In vitro	Motor		Motor	Motor		Inhibit.**	Ambache and Hammond (1949)

* Occasionally the inhibition effect was not preceded by a motor response.

** A diphasic response involving a motor effect followed by inhibition was observed also.

Interpretations of the effects of epinephrine on uterine activity must be made with caution, for some investigators have called the effect inhibitory when their tracings actually show a diphasic response. This may be responsible for some of the disagreement with respect to other species as well. Dosages used also may affect the results. Reynolds (1949) has pointed out that, in some cases, small doses may constrict the arterioles of the uterus without affecting the myometrium directly. He also indicates that varying responses to epinephrine may be caused by the pH and the calcium content of the fluid used. These all indicate the need for caution in the interpretation of results.

THE ROLE OF MOTILITY IN THE FEMALE GENITAL TRACT

Various roles have been assigned to the activity of the female reproductive tract. The type of activity shown near the time of ovulation and for a time thereafter has been considered to play a part in the transport of the ova. The literature bearing on this subject has been reviewed by Hartman (1932, 1939). Recently the interests in the Department of Dairy Science at the University of Illinois have turned to the possible role of the reproductive tract of the female in sperm transport. The remainder of this review is devoted to a discussion of factors pertaining to sperm transport in the female.

MOTILITY OF THE FEMALE REPRODUCTIVE TRACT AT MATING

An early suggestion that the activity in the female reproductive tract is associated with spermatozoan transport was made by Heape (1898). His findings have been referred to by a number of workers and he has mentioned that motility of the uterus was induced by stimulating the erectile tissue of the vulva. Westman (1926) observed that bringing a male rabbit into the presence of the female caused the genital organs to become hyperemic and increased contractions took place in the tubes. The hyperemic condition soon disappeared following the removal of the male but the strong contractions continued. In the following period the contractions gradually decreased but were renewed if the male was brought into the presence of the female again. Westman found that this response to the presence of the male came almost immediately. Heape's observations concerning the stimulation of activity of the reproductive tract through stimulation of the vulva was confirmed by Krehbiel and Carstens (1939) when they found that various fluids placed in the vagina of the rabbit would be carried throughout the uterus, providing artificial stimulation to the vulva was applied. Reynolds (1930) recorded increased uterine activity in the rabbit following mating. In contrast to these findings with the rabbit, Bickers and Main (1941) reported that uterine motility was temporarily abolished following coitus in the human. The recordings which they present were made approximately two hours following coitus. Others who have recorded increased activity at mating have made observations at or soon after mating.

A study of the effect of mating on the activity of the uterus of the cow was made by VanDemark and Hays (1951, 1952). These investigators have reported that various stimuli of natural mating including the sight of the bull, nuzzling of the vulva and rear quarters by the bull,

non-copulatory mounting, and finally copulation all produced uterine contractions within the cow. The response to copulation and ejaculation was the most marked of those shown as a result of the various stimuli. An example of uterine activity in the cow during natural mating is shown in Fig. 2(2). These investigators also reported that such responses were shown by both estrous and post-estrous cows with the greater response being shown by those cows in estrus.

VanDemark and Hays (1951) have shown also that various stimuli associated with the artificial insemination of cows produced contractions in the bovine uterus [see Fig. 2(1)]. Activity of the reproductive tract produced by mating and artificial insemination is due in part, if not entirely, to the release of oxytocin brought on by these procedures. Neusch (1904) pointed out that the manipulation of the reproductive tract of the lactating cow would cause the ejection of milk. Neusch cites references to reports in 1727 which indicate that a technique of blowing air into the reproductive tract of the cow and mare was used by certain tribes of India and Africa to bring about the milk ejection process in the absence of the calf or foal. Hammond (1936) observed that, in lactating mares, milk often flows freely during the mating process. Harris (1947) has suggested that oxytocin is released during mating in the rabbit. Conclusive evidence for the release of oxytocin or an oxytocin-like substance during natural mating and artificial insemination of the cow was obtained by Hays and VanDemark (1951, 1953d). These investigators found in 88 per cent of 99 trials made on 16 cows that artificial insemination techniques resulted in an increase in intramammary pressure. Natural mating likewise caused a positive response in 80 per cent of the trials with 15 animals. No significant differences due to various stages of the estrous cycle were evident in the increase in intramammary pressure resulting from the stimulation of artificial insemination techniques.

UTERINE ACTIVITY AND SPERMATOZOAN TRANSPORT

Since it is evident that oxytocin is released at the time of mating or artificial insemination and this is the cause of increased uterine activity during these procedures, and since the speed of spermatozoan transport in the cow (VanDemark and Moeller, 1951) and in several other species is more rapid than can be accounted for by the motility of the sperm themselves, the involvement of uterine activity in spermatozoan transport seems evident. In fact, Hays and VanDemark (1952) have demonstrated that sperm transport can be brought about in a matter of minutes in the perfused excised reproductive tract of the cow if oxytocin is added to the perfusate at or soon after the deposition of semen into the cervix. When oxytocin was not added, sperm transport did not occur even when as much as 30 minutes time was allowed before examination. Numerous difficulties have been encountered in the attempts to study sperm transport *in vitro* and these studies are still in progress. While all trials have not resulted in the successful transportation of sperm from the cervix to the oviducts, there is no doubt that oxytocin additions and the resulting uterine contractions result in carrying sperm throughout the uterus and in some cases throughout the oviducts.

Since uterine activity is responsible, in part if not entirely, for sperm

transport, the question arises as to whether or not the injection of oxytocin at the time of mating would increase spermatozoan transport and result in improving fertility. Hays and VanDemark (1953c) have found that oxytocin injections into cows immediately following natural mating has resulted in a significant increase in conception rate over control animals receiving no injections. These investigators found that epinephrine injections preceding natural mating also resulted in increased conception in dairy cows. No explanation for this result has been offered. However, preliminary results have indicated that the injection of epinephrine prior to mating resulted in reduced uterine tone, but the stimulus of natural mating overcame the epinephrine inhibition and resulted in uterine contractions similar to those observed normally. Perhaps these produced greater sperm transport than occurred in the control animals. This point remains to be proven. The stimulatory effects of artificial insemination techniques on uterine activity in the cow seem to be more easily blocked by epinephrine than are those associated with natural mating. Further evidence on the effect of oxytocin and epinephrine injections at the time of artificial insemination is being collected.

It appears that the way an animal is handled at the time of breeding may influence fertility in dairy cows by affecting uterine activity and sperm transport. This emphasizes the necessity for the proper training of inseminator technicians so that careful techniques are used. Furthermore, the careful handling of animals at the time of natural mating seems important. Whether similar conditions exist in other animals remains to be seen.

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PRENATAL DEATH AS A FACTOR IN THE FERTILITY OF FARM ANIMALS[1]

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The fertility of a given species may be considered as a function of three variables: female fecundity, fertilization rate, and prenatal death rate. Brambell (1948) stated that there is no satisfactory estimate of the total prenatal mortality from fertilization to birth for any single species of mammal, not excluding man.

Recognition of the importance of prenatal death in livestock production was first given special emphasis by Hammond (1914). He found on the basis of seven sows in various stages of pregnancy that the number of normal fetuses was 73 per cent of the number of corpora lutea present in the ovaries. These data were increased in his 1921 report and on the basis of 22 sows the proportion of normal fetuses was 67 per cent; the fetuses that were atrophic, 12 per cent; and those missing entirely, 20 per cent. He also presented data on 80 pregnant ewes which showed normal fetuses equal to 87 per cent of the corpora lutea present. The number of atrophic fetuses and missing eggs were approximately equal.

Corner (1923) studied the problem in swine and gave some recognition to the importance of stage of gestation in connection with the estimation of embryonic death. He was puzzled by an apparent loss in his packing-house material of 40 per cent of the embryos in the first few days of gestation whereas in later stages only 20 to 30 per cent of the embryos appeared to be degenerate or missing. His explanation assumed that some sows which would appear at earlier stages as having both normal and abnormal embryos would finally lose all their embryos and thus would not be recognized as having been pregnant in later stages.

Henning (1939) estimated the incidence of fetal mortality in sheep from the discrepancy between the number of corpora lutea in the ovaries and the number of live fetuses in the uteri. He pointed out the fact in his material that early embryonic death with complete absorption could not be detected. Over-all he found 16 per cent of the corpora lutea were not accounted for by live fetuses, a figure which is but slightly higher than that noted earlier by Hammond.

Henning called attention to the increase in the mortality of the fetuses with the increase in number of ova shed: 8 per cent with one ovum, 26 per cent with two, and 43 per cent with three. His material might just as

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well be interpreted that with a large number of eggs more embryos must die before the pregnancy is lost, even though the probability of death for an individual embryo was uniform. The increase in mortality may then be more apparent than real.

Estimates of embryonic death in cattle, where the rule is for one egg at a time to be ovulated, would seem almost valueless when made from packing-house material. Complete loss of the embryo makes the cow indistinguishable from an unbred animal, and the mortality is not detected in the estimate of embryonic death. Complete litters also could be lost as a chance deviation in a litter-bearing animal such as the sow, but such an occurrence should be exceedingly rare if the probability of death is fundamentally the same in one sow as in another. If the embryonic death rate were 0.5, and if the average litter size were 12, the probability of complete loss would be 0.5^{12}, which would be extremely rare. Brambell (1948), however, has pointed out that in the wild rabbit the probability of death differs from litter to litter, and more will be said about this later in connection with swine.

It has become apparent that an estimate of embryonic death requires an embryo-census at two successive stages of gestation and perhaps on two separate but entirely comparable groups of animals, e.g., immediately after fertilization and again at term for complete measurement of pregnancy wastage. The determination shortly after fertilization gives an estimate of the ovulation rate and of the fertilization rate. It is assumed then that the animals studied at parturition had the same number of eggs ovulated and fertilized as was determined in the first group.

Re-evaluation for swine of the proportion of corpora lutea accounted for by young born at term was made by Casida (1951) as approximately 56 per cent. It was based on the comparison of similar groups of females, one killed shortly after fertilization and the other allowed to farrow. Squiers et al. (1952) made a check in a similar manner of the losses during gestation and estimated that 54 per cent of the eggs ovulated are represented at term by living pigs. The estimates are lower than expected from Corner (1923) who stated that about 70 per cent of the ova are represented at term by living pigs. It should be remembered, however, that his estimate was based on packing-house material.

Perhaps a certain amount of embryonic death should be viewed as normal. The complexity of the developmental process, from fertilization to parturition, and the infinite number of steps at which accidents of development may occur leads us to give this point of view sober consideration. Any increase in embryonic death would be a major factor in lowered fertility.

A typical kind of lowered fertility is the "repeat-breeding" of females of all classes of livestock. No obvious cause is evident for the failure of such females to conceive, but repeated breedings to "normal" males fail to produce conception. Particular emphasis has been given this condition in the artificial insemination program with dairy cattle. The problem has often been analyzed as one of fertilization failure. Poor semen quality, defective artificial insemination technique, toxic conditions of the female's genital tract, low grade infections, etc. have all been suspected. An analysis was made (Tanabe and Casida, 1949) of the reproductive failure in such cows into the two components, fertilization failure and embryonic

death. The fertilization rate observed in that study, approximately 60 per cent, appeared high in relation to the usual non-return rate observed at that time. Furthermore, the loss of approximately two-thirds of the embryos within the first 34 days after breeding made embryonic death appear to be a major factor in such cases. No controls were available in that study.

Abnormalities of fertilized bovine ova were shown by Winters et al. (1942), and they called attention to the possible role of such abnormalities in lowered fertility. Embryonic death had been demonstrated as in an portant cause of lowered fertility in cattle by Laing (1949). He had data also on 11 maiden heifers, inseminated to a single bull of known high fertility, which showed 100 per cent fertilization. His observation was at least suggestive that the normal fertilization rate is high.

An attempt has since been made to determine the fertilization rate in normal first-service heifers under the conditions of artificial insemination and to relate this to the non-return rate in the field so as to estimate embryonic death. A comparison further has been made of the poorer and the better bulls used in artificial insemination to see if differences in their fertility level is attributable to fertilization failure or to embryonic death. These estimates (Kidder et al., 1952, 1952a, and unpublished) indicate over-all that the fertilization rate is approximately 86 per cent and that the embryonic death rate in the first 60-90 days is approximately 30 per cent. (This estimate probably is high inasmuch as it assumed no incidence of anatomical defects preventing fertilization in the field cows.) When the data are analyzed so as to determine the difference between the better and the poorer bulls, the fertilization rate on those bulls with non-return rate of 67 per cent and more is approximately 100 per cent. The poorer bulls had a fertilization rate of 72 per cent. Embryonic death rates were found of approximately 35 and 25 per cent respectively, which gives no real indication that the bulls of the two levels of fertility actually differ. If the lowered fertility of the poorer bulls is brought about by some infectious organism carried by the semen and which is active, despite the antibiotics used in the dilutor, the embryonic death rate might be expected to appear higher in the poorer bulls. No evidence of this was obtained for such bulls as a class. Further than this, the very high fertilization rate observed tends to point out that the repeat-breeding cow probably does have an increased failure of fertilization as well as increased embryonic death. Antibiotics, however, have been pretty generally introduced in semen dilutors with their concomitant improvement in fertility since the time of the initial study of the repeat-breeding cow. Thus the actual fertilization rate in such cows as determined with antibiotic-treated semen may be higher than observed in that study. Tanabe and Almquist (1953) have recently determined the fertilization rate in repeat-breeding heifers using antibiotic-treated semen and found it to be 70 per cent, or a little higher than found in the earlier cows without antibiotics.

An estimate of embryonic death in sheep has been obtained in a preliminary analysis of the contribution of fertilization failure and embryonic death to early season infertility (Voigtlander and Hulet, University of Wisconsin, unpublished). Western ewes were the test animals for determining fertilization rates throughout the breeding season, the numbers of

test animals studied in each part of the breeding season being fairly proportional to the number of ewes bred in the breeding flock at the same time. The test animals were killed so that an estimate could be made of the fertilization rate in the different parts of the season and this in turn could be related to the lambing rate from breeding in the different parts of the season. An over-all determination of embryonic death on the basis of 18-day non-returns was 20 per cent, but on the basis of lambing rate was 30 per cent. This estimate, like that for the cow, may well be high because it assumes no anatomical defects causing fertilization failure in the flock ewes.

Estimates for the three classes of farm animals that have been presented, although only roughly comparable, raise the question whether or not embryonic death may not be higher in litter-bearing animals than in single-bearing. Estimates made for the rabbit, both wild (Brambell, 1948) and domestic (Casida, 1951), appear to be in line with swine, i.e., 35 to 45 per cent. They appear as a class to be in contrast with the situation in cattle and sheep, where it is more nearly 30 per cent.

Robertson et al. (1951) investigated pasture, protein level, and feeding level as factors that possibly affect variation in the embryonic death of swine. Limited effects only were noted for pasture and for level of protein. The most general effect was from full feeding. The percentage of eggs resulting in normal embryos at 25 days gestation was less by 25 per cent in those animals that were full fed.

Christian et al. (1952) obtained similar results. They compared gilts that were on high and low planes of nutrition. The estimated prenatal death rate in the high plane animals was greater by 27 per cent than in the low plane animals. They found, as did the Wisconsin workers, that even though a higher ovulation rate was obtained by full feeding, the greater embryonic death resulted in smaller litters than from the gilts on the low plane of nutrition.

Preliminary studies of Self (unpublished, University of Wisconsin) are consistent with the earlier work on embryo survival; on limited feeding it was greater by 25 per cent than on full feeding at 25 days of gestation. His data are also suggestive that the "fatness" of the animal during early gestation (level of feeding prior to breeding) may not be as important as the level of feeding at the time of early pregnancy itself. Changing from limited to full feeding one estrual cycle before breeding resulted in a decrease in embryo survival of 18 per cent. Changing from full to limited feeding at a similar interval before breeding resulted in an increase in survival of 16 per cent.

Other evidences of the effects of feeding upon embryonic death in swine are given by Moustgaard (1952). Animals that were fed animal protein or plant protein plus B^{12} had embryonic death rates at four weeks of gestation of approximately 20 to 30 per cent. Plant protein without B^{12} gave embryonic death rates of approximately 50 per cent. The experiment is cited by Møllgaard (1952) as an instance of complete subclinical vitamin deficiency, not affecting the mother in any clinically detectible way but having a deadly effect on the offspring.

Squiers et al. (1953) reported on the effects of inbreeding upon embryonic death in swine. They estimated that for each 10 per cent of inbreeding of the parent lines the advantage for line cross sows amounted to 0.33

more pigs at 25 days gestation although both inbred and linecross animals were carrying outbred pigs. They also pointed out that embryonic death was of more importance in controlling litter size in swine than ovulation rate. The standard partial regression of litter size on ovulation rate was 0.79, whereas on mortality rate it was -0.94. Similar studies by Baker (unpublished, University of Wisconsin) confirm the relative importance of the two factors with values of 0.74 and -0.96, respectively. Baker studied potential embryo loss at 25 days and also at 70 days of gestation in swine. The values at these two stages were 22 and 45 per cent. Differences between sows in the proportions of missing or dead embryos were significant at both stages. Estimates of the amount of sow variability showed it to be essentially the same at 25 days as at 70 days (72-82 per cent) despite the fact that much additional mortality had occurred between 25 and 70 days.

The analysis of potential causes of embryonic death made by Hammond in 1914 tended to eliminate disease as a factor. He believed this was true because dead and live embryos could exist side by side in the same uterus. Bacteria usually were not found and no particular pathology was usually present in the maternal tissues. The general nutrition of the mother did not seem to be a particular factor because much mortality occurred before embryos were large enough to be serious competitors for any restricted nutrient, and further he saw little relation between numbers of embryos in the early stages and amount of embryonic death. (Little or no evidence in the earlier stages of gestation was found either by Squiers et al., or by Baker.) Hammond concluded that something inherent in the egg seemed the most likely cause. He suspected that the larger the number of eggs produced by either ovary, the greater the amount of embryonic death, and interpreted it that ovarian nutrition was a limiting factor for the production of eggs capable of resulting in viable embryos. This hypothesis would at least be consistent with there being litter differences in embryonic death and also with the suspected difference between single and litter-bearing species in the amount of embryonic death. Baker, however, examined his material for evidences of an association between the largest number of corpora lutea in either ovary and embryo losses but found none of significance.

Explorations have been made of possible genetic, nutritional, and disease factors in embryonic death in cattle. These studies, usually on repeat-breeding cows, have for the most part been controlled tests of empirical treatments. Crossbreeding (Christian et al., 1951, Corley et al., 1952) increasing the blood ascorbic acid level (Christian et al., 1951) and intrauterine infusion of penicillin, streptomycin, and aureomycin (Ulberg et al., 1952) all have failed to give evidence of decreasing it.

We are in the stage of searching for associated phenomena -- variables that have the potential in themselves of being causes of embryonic death or of leading us to the causative factors. Little has been found that is suggestive. Asdell et al. (1942) and Bentley et al. (1951) studied the blood chemistry of repeat-breeding cows but found little that was unusual. The most promising associated phenomenon to appear thus far is one that is not always consistent; it is, however, uniform enough to demand further explanation. The defense mechanism of the uterus of the repeat-breeding cow seems to differ from that of a first-service animal.

Insemination into the uterus of repeat-breeder cows or of first-service heifers all in the luteal phase of the estrual cycle has resulted more consistently in a pyometra of the first-service animal than it has of the repeat-breeder (Black et al., 1953). Further it has been shown that controlled in vivo culturing of an organism E. coli in the uteri of these two kinds of cattle has resulted in a larger number of organisms in the majority of first service animals than in paired repeat-breeders (Black and Simon, unpublished, University of Wisconsin). One interpretation of these results is that the repeat-breeder tends to exert greater bactericidal or bacteriostatic activity than the first service animal.

A poorer bacterial defense mechanism of the uterus in the luteal than in the follicular phase of the estrual cycle has been demonstrated in cattle, swine, and rabbits. It has been demonstrated by Black, et al. (1953, and unpublished) that the uterine defense against infection is approximately as great in the ovariectomized animal as in the estrogen-treated. Progesterone, on the other hand, appears to block the natural defense mechanism of the tissues. It seems more than a coincidence that the ability of the uterus to defend itself should be made low particularly at the time the embryo is establishing intimate nutritive arrangements with the endometrium. The possibility, supported by some evidence that the defense mechanism of the repeat-breeder uterus is not rendered so passive in the face of attacking agents as is the more-probably-fertile first service animal, needs further exploration. Inhibition of natural endometrial defenses may be necessary to permit normal placentation. Progesterone from the standpoint of its production, destruction, and excretion, and also the responsiveness to it of the target tissues is definitely indicated for study. Whether or not the fundamental underlying increased embryonic death as found in the inbred gilt or in the full-fed gilt or in the repeat-breeding cow lies in this complex, or whether basically different causes are operative remains to be determined. The complex would appear, however, to be one whose potential derangements would satisfy the requirements for the theoretical causes of embryonic death as we know them at the present.

The success of an embryo in its development may depend on its surviving a series of hazards and these hazards may be accidents of development or they may be genetically determined weaknesses in certain developmental steps. The fact that there are maternal differences in embryonic death rate gives emphasis to the study of the maternal environment itself even though as a rule only a part of the embryos perish. The chief variability in the character would appear to be in the maternal threshold for bringing about its expression, i.e., for converting the accidents or the weaknesses of development into embryonic fatalities.

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FACTORS INVOLVED IN STERILITY OF FARM ANIMALS

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The problem of sterility and infertility in farm animals is a large one and involves considerable economic and time loss to farmers. An estimate I made some years ago was that in all its forms it represented an annual loss of nearly a quarter of a billion dollars to the farmers of the United States, and I do not suppose that this figure is any less now.

My remarks will be concerned mainly with infertility in dairy cattle as this is the species with which I have had most contact. Some of my remarks may, no doubt, be extended to other species, but I have become wary of generalizations without the firm support of factual observations. The relative emphasis to be placed upon the various factors involved differs considerably, as I shall endeavor to point out. During the past few years we have learned a good deal about the various phases of the problem, especially since the technical advisory committees of the Research and Marketing Act have been operating. Just about that time an excellent symposium on the general subject was held at Athens, Georgia, during the meeting of the American Dairy Science Association. This included papers on the disease aspect (Bartlett, 1949), hormones (Asdell, 1949a), nutrition (Asdell, 1949b), inheritance (Gilmore, 1949), and on methods of approach (Sykes, 1949). A systematic attempt to solve several of the outstanding problems outlined by Sykes has been made and considerable progress can be reported.

All the aspects mentioned above are important, but there is much that can be done by the farmer himself to improve the breeding capacity of his livestock. The work of the New York Mobile Laboratory has shown that faults of management play a large part in the infertility problem. Among those which have been uncovered in this work the following stand out:

(1) Failure to detect signs of heat together with improper timing of services. Laing (1944) has emphasized this also, while Roark and Herman (1950) have made a detailed study of estrus and its detection.

(2) Breeding back too soon after calving. Sixty days is soon enough as Shannon et al. (1952) have shown.

(3) Failure to keep breeding records. This is particularly important in herds with more than one bull. We have come across cases in which the owner did not detect a low fertility bull as soon as he should.

(4) Switching bulls when a cow does not settle to the first one used. This is a good way to spread infection. We have good evidence that vibrio fetus infection has been spread from bull to bull and from herd to herd by this practice.

(5) Failure to have regular pregnancy checks. Various surveys have shown that from 40 to 60 per cent of cows slaughtered for sterility were

actually in calf at the time of slaughter. Only part of this can be due to cows which come in heat during pregnancy. Donald (1943) reports that it occurs in at least 3.4 per cent of cases.

(6) Failure to call the veterinarian early in cases of infertility. We consider that an examination is called for in all cases of absence of heat, of more than 3 services without conception, in all cases of unnatural discharges, and in cases of heat at intervals of less than 15 days. The earlier a veterinarian can work on a cow the better chance of success he has, and he can also prevent the possible spread of disease by suggesting suitable methods of breeding hygiene.

All in all, we have still a great deal to do in educating the farmer to help himself. It may amount to half the breeding problem. I do know that where we have a dairyman who takes a real interest in his cows, who can go down the line and give a detailed history of each cow and of her dam, we have often a different type of infertility than is the case with the dairyman who does not know his cows. One man is a close observer and usually efficient. His trouble is more likely to be a straight disease problem if he has one at all.

In our mobile laboratory work we have encountered our full share of disease problems and structural abnormalities. Some of the latter may be inherited but we have no clear-cut observations on this possibility. Disease and tumors are found in wide variety but individually most of them account for a very small proportion of total infertility. Brucellosis is no longer a major problem; vibriosis has taken its place in our thoughts. We are still in the initial stages of research on this disease. We agree with Plastridge (1951) that abortions do not account for the most of breeding troubles in this disease. Rather, delays in breeding take the larger toll. We have learned to associate a certain type of breeding behavior with this infectious disease. This consists of several matings at the usual 21 day interval or a few days longer, then an interval of 2-4 months, one or more matings, and a calf carried to term. When we encounter several records of this sort in a herd we strongly suspect vibrionic infection and look carefully for further evidence. I also believe that, in herds with chronic infection, cystic ovaries and an undue proportion of twin births are frequent. In this connection an observation by Clapp (1934) some years ago of a herd in which cystic ovaries and twin births were associated may be significant.

We have much to learn about vibriosis. I am not satisfied that all the vibrio-like organisms we observe under the microscope are pathogenic. In England it is strongly suspected that this is the case. The organism seems to exist in several strains of varying pathogenicity and one strain appears to vary much from time to time, at least in its agglutination properties. We need much more work upon the organism itself as well as upon the disease it produces. The problem is a serious one since both in America and Denmark about one-third of A. I. bulls are said to carry the organism. In Sweden I also found considerable concern about it. In England, while the danger is fully recognized, I found that there was a tendency to discount its importance for the reason given above and also because some of the workers are inclined to give prior position to an organism of the pleuro-pneumonia type (See discussion by Hignett in Asdell, 1952).

We have found but few instances in which infertility could be put down to trichomoniasis infection. This is in contrast to the position in Holland and Belgium in which countries it is considered to be a major problem.

Nodular vaginitis, or vulvitis, seems not to impair fertility, at least not to any marked degree. Yates (Anon. 1953) of Rhode Island and Olson (Anon. 1953) of West Virginia have studied this problem intensively, and this is the conclusion that may be drawn from their work.

The relationship between inheritance and fertility is confusing. The work of Bartlett and Pfau (Anon. 1952) at New Jersey and of Pou and Henderson (Anon. 1952) at Cornell show very low hereditability of services per conception and calving intervals in dam-daughter comparisons. The relationship is so low, very near zero, that we do not recommend selection for fertility if selection for more highly inherited traits, such as milk and fat yield, suffer in the process. The figures are for cows that do conceive and are mass data. They mean that, in this class of cows, inheritance plays but a small part in the over-all infertility picture. They do not deny the existence of inherited infertility in a limited number of individual cases, nor do they deny the inheritance of factors that may cause absolute sterility. When I was discussing this problem in Norway and Sweden, strong exception was taken to the views I have put forward here. In those countries, clear evidence of infertility due to hereditary factors has been uncovered, especially of genital hypoplasia by Lagerlöf and Boyd (1952). I think that the difference in our viewpoints is that we have been dealing with large breeds with little or no linebreeding or inbreeding, while the Swedish workers have obtained their data from more restricted breeds that are much more linebred than ours. This has led to the concentration of factors causing infertility. In this country the existence of such genes has been shown by the inbreeding work of Mead et al. (1946) in which factors affecting both male and female fertility have been uncovered. It would seem, at present, that as much information as is possible has been obtained from the treatment of mass data, and that further progress will have to be made by isolating and experimenting with strains in which inherited infertility factors can be recognized.

Lethals form another small part of the infertility picture as they cause the formation of unviable zygotes, thus limiting breeding efficiency. I have come across a bull that carried two lethals. The results of his use in a linebreeding program may well be imagined.

Nutrition is another aspect of the problem and it is probably the field in which anecdotal lore has obscured the true picture most. When all other explanations have failed to account for infertility, malnutrition has been blamed. The experimental literature has been reviewed by Reid (1949). For cattle, I think that the general position can be summarized as follows: Most failures are due to over-all underfeeding, often with poor quality feeds, so that deficiencies are multiple, not specific. Before reproduction suffers, the animal shows signs of the malnutrition. The amount of feed actually used by the reproductive system is small compared with that used by the animal as a whole. Semen and ova contain very little dry matter and, during pregnancy, the cow puts on about 6 per cent of her live weight in 9 months, a small amount compared with her rate of growth as a heifer. The position may be different for the pig as her progeny represents a much greater proportion of her live weight.

There is no evidence that requirement by the reproductive tract for any specific substance in the feed is greater than it is for any other tissues. When the malnutrition impairs the orderly development of the reproductive tract during early life, the damage is more apt to be permanent than it is if the damage occurs after the tract has once functioned in a normal manner.

Calfhood malnutrition of a relatively mild nature delays reproductive development but does not wholly suppress it. The Cornell work, still in progress, shows that puberty is delayed but that ovulations do occur eventually. The eggs shed have the same chance of fertilization as do those shed in properly nourished heifers but parturition difficulties are great due to the poor development of the mothers.

Functional sterility is another "catchall" for poorly understood cases of reproductive failure. It should represent cases in which hormonic outputs, balances, or sequences are out of gear. Such cases are difficult to prove and we still await accurate assay methods for the hormones concerned. Eventually we shall be able to point to these functional sterilities as results of other defects; they may be the immediate causes of infertility but they, in themselves, have their causes. We are beginning to understand these. Impaired nutrition seems to reduce the secretion of follicle stimulating hormone by the anterior pituitary. This, in the young animal, prevents full ovarian development. In the adult, it causes failure to develop graafian follicles and ovarian atrophy with all its consequences on the rest of the tract. Climatic factors may act in the same way, though in the sheep there is evidence that they regulate the release of luteinizing hormone, the factor causing ovulation, from the anterior pituitary. Kammlade et al. (1951) have shown that the gonadotrophic hormone content of the anterior pituitaries of anestrous sheep is as high as it is during the breeding season. Large follicles are present at this time also. It is tempting to assume that the fault lies in non-release of luteinizing hormones though the occurrence of ovulations without estrus before the time the breeding season begins is puzzling.

Continued secretion of F.S.H. without change to L.H. secretion would seem to be a cause of cystic ovaries and nymphomania. The balance of evidence suggests that this may be a consequence of overstimulation with estrogens since the condition occurs in the cow when estrogens are implanted. It also seems to occur in some conditions involving uterine pathology. We still have much to learn about the relationship between the endometrium and the pituitary or ovary.

Persistent corpus luteum is often cited as a cause of absence of heats, and this is logical. But I do not know of any good criterion for a diagnosis of persistent corpus luteum. Deep seated corpora lutea are usually regarded as persistent, but I do not know of any cases in which their history has been followed sufficiently carefully to confirm the diagnosis. We need more information on the condition.

In discussing functional sterility, it must be pointed out that the cow is extremely sensitive to the hormones concerned in reproduction, more so than any other of our domestic animals. This increases the difficulty of working with them and emphasizes the need for caution in their clinical use. We urgently need methods of assay sufficiently accurate for use upon the small quantities present in the tissues of cattle.

Most of my remarks have been devoted, as I have already said, to conditions in cattle. A few general remarks about conditions in other species may not be out of place as this conference takes into account all farm animals, though my experience with the other species is limited.

In the horse, the chief reproductive difficulties seem to be in the development of cystic and fibrotic ovaries. These may be due to the notorously irregular breeding of mares, in other words, they may be the results of management practices. The long heats and apparent difficulty of handling the spermatozoa are other difficulties that have to be considered.

In swine, I have encountered many cases of imperfect development of the uterus, blind and missing segments, and this seems to have been the experience of Wilson, Nalbandov and Krider (1949). I have also been impressed by the number of cases I have encountered of blood follicles and premature luteinization of follicles with consequent imprisonment of the ova. True hermaphroditism is of more frequent occurrence than it is in other species, and it may have an hereditary origin, but it is not frequent enough to be a major problem.

In sheep, I should be inclined to give first place to ovulation without heat and failure to develop ripe follicles. But infertility in sheep has had remarkably little attention paid to it.

In goats, a major factor is the large amount of pseudo-hermaphroditism encountered. These are genetic females and the gene producing them acts as a simple recessive. It is probably extremely closely linked with the dominant gene for hornlessness as horned pseudo-hermaphrodites are extremely rare (Asdell, 1944). Since I wrote this note, the suggestion has been confirmed by Eaton (1945) but there is more to the problem yet. O'Mara (1945) has commented on the relative absence of homozygous polled goats; he could only cite two in the United States. This is so, as I have been able to find very few in the records of the British Goat Society. Examination of their records revealed 3, or possibly 5, in a sample that should have contained 22. Stenosis of the epididymis is frequent in bucks; can one hazard the suggestion that the missing homozygous dominants are in this class? Anyway, pseudo-hermaphroditism may be eliminated in goats by breeding only horned animals.

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PROBLEMS IN THE FIELD OF PHYSIOLOGY OF REPRODUCTION OF FARM ANIMALS

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Though the title assigned me might infer that my paper was to be a summary of those preceding, an attempt will not be made either to summarize the papers which have been presented or to fill in any gaps. Rather, my views will be presented on some methods which might be productive in the solving of the various problems which confront us. Before doing this, however, some of the more important problems will be enumerated: embryonic deaths, failure of estrus, failure to ovulate, variation in time of ovulation, delayed parturition, cystic ovaries, delay of sexual season, failure of estrus and ovulation during lactation, small litter size, metabolism upsets during lactation, lack of sexual libido in the male, lack of sperm, abnormality of sperm, abnormal male accessory organs. Then there are a number of man-made problems such as problems in egg transfer, problems related to artificial insemination of cattle, etc.

Now let us consider how one might proceed to solve some of these problems. All of my suggestions involve tedious work and, in many cases, more skill than many of us in this audience possess--that is, more skill as regards training in specialized fields, such as steroid or protein chemistry for example.

Morphology of the reproductive organs and of the gland controlling them.

It is my impression that there is still much to be learned by a more detailed study of the morphology of the reproductive organs and of the glands controlling them in normal and abnormal animals. The classical studies of Tanabe and Casida (1949) on the morphology of embryos in early stages of development can be cited as an example of contributions needed in this field. The studies of Hooker and Forbes (1947) on the cytological effects of progesterone in the uterus, which made it possible for them to develop a new method of progesterone assay, is another example. The more recent finding of Olsen, Salhanick, and Hisaw (1952) that the connective tissue response in the uterine mucosa to progesterone is inhibited by certain estrogens does not make these studies unimportant. Unpublished histological studies by Cupps in our laboratory have led to the conclusion that the adrenal cortex may be involved in certain types of sterility. In this connection, Plate (1952) has reported that chorionic gonadotrophin increases the output of 17 ketosteroids by the adrenal cortex in castrate human subjects.

Hormone levels in glands, body fluids, and in excreta

Very little is known about the concentration of hormones either in the glands producing them, in the body fluids, or in the excreta during different reproductive states. In some instances, it will be necessary to devise new techniques in order to accurately determine hormonal level, particularly in body fluids and in excreta. More sensitive biological assay methods will perhaps facilitate studies of hormonal levels. This is particularly true of the protein hormones which are difficult to isolate chemically. As an example of the magnitude of the problem, our studies pertaining to gonadotrophins in the non-pregnant mare may be cited (Cole and Goss, 1939). These studies indicate that it takes approximately 500 to 1000 milliliters of blood serum to represent one rat unit. If it is necessary to draw two liters of blood in order to obtain sufficient hormones to produce a physiological effect in one rat, it is obvious that one will not be able to accurately determine the hormonal levels at different phases of the estrous cycle. Consequently, it will be necessary to devise sensitive chemical or biological tests which necessitate the drawing of less blood before this particular problem can be tackled. Awaiting the development of such tests, progress can be made by studying in detail the hormonal levels in the secreting gland at various reproductive stages.

It is difficult, however, to interpret the potency of the pituitary in terms of secretory rate. Robinson and Nalbandov (1951) studied the gonadotrophic potency of swine pituitaries throughout the estrous cycle and Kammlade et al. (1952) made similar studies in ewes. In both species the gonadotrophic potency of the pituitary increased as the stage of the cycle advanced. Nonetheless, it is likely that the most rapid secretion of ICSH occurs during estrus. Furthermore, Kammlade (1951) found the greatest concentration of gonadotrophin in the pituitary during anestrus, a period in which one might expect decreased secretory rate. The authors postulated that FSH secretion is high and ICSH secretion low during anestrus.

It is possible that the hormonal levels in the urine could be determined, but here again the relatively low concentration would probably make it extremely difficult with the biological tests now available. In the case of the steroid hormones, chemical procedures, more specifically chromatographic procedures, look very hopeful as a means of determining the levels of these substances in glands, body fluids, and excreta. However, I feel that it will also be necessary to make extensive studies on the exact nature of the steroid substances present in tissues and fluids of domestic animals. This stresses the desirability for more steroid chemists to be concerned with these problems. It is obvious that steroid chemists associated with physiologists working on large domestic animals would greatly facilitate this particular study. Progress on this problem to date has been summarized by Cole (1950). Chromatographic methods may also be useful in determining levels of protein hormones (Taurog, Briggs, and Chaikoff, 1951).

As yet, satisfactory evidence is lacking for the secretion of LH. D'Amonr's studies with human urine did not indicate any qualitative differences in the gonadotrophin content during the menstrual cycle. Rather, the qualitative response appeared similar to that of menopausal urine gonadotrophin and the gonadotrophin in the urine of man. Definite evidence

s available that pituitary gonadotrophin content varies qualitatively in different reproductive states, but this could be explained on the basis of the presence of varying amounts of "prehormone". Thus an intensive search should be made in body fluids and excreta for a substance resembling LH as obtained in pure form from the pituitary.

Physiological effects of hormones

No doubt there is still much to be learned regarding the physiological effects of hormones both singly and in combinations. For example, the original studies of estrogens by Allen and Doisy indicated that estrogens had an effect upon the accessory reproductive organs of the female and upon sexual receptivity. We recognize now that the estrogens influence many other glands and issues. They have an influence upon the pituitary gland regulating the secretion of its hormones, they influence the growth of the epithelium of the skin, they influence bone growth, they are concerned with phospholipid metabolism, and in ruminants they have a very pronounced effect upon the development of the carcass. Our studies lead us to conclude that these growth effects on ruminants are indirect ones, dependent upon an increased secretion of androgen by the adrenals which, in turn, is stimulated by an increased secretion of ACTH by the pituitary gland. Estrogens have an ameliorating effect upon the psychological disturbances associated with the menopause. Though I shall not enumerate them, androgens likewise have an effect upon many activities in the body. Intensive studies of a similar nature are needed for others of the hormones. For example, relaxin has received very little attention. We know that it has an influence upon the pubic symphysis but other physiological effects are undetermined. It is very likely that relaxin has a much more widespread effect than those which have been determined to date. Furthermore, a more detailed understanding of the physiological effects of hormones may help in solving problems in reproduction.

Little is known about the secretory rate of the hormones in the various domestic animals. Studies of secretory rates of gonadal hormones by comparing the concentration in venous and arterial blood should be particularly fruitful. Much has been added to the knowledge of the physiology of the adrenal cortex in this manner (Paschkis et al, 1950; Busch, I. E., 1953).

New hormones

One should constantly bear in mind the possibility that there are still many hormones which have not been discovered. One need only to refer to the studies in recent years on the adrenal cortex to emphasize this particular point. Some twenty-odd steroid substances have been isolated from the cortex, and evidence is now available that a number of these are actually secreted into the bloodstream. It is true that physiological activities have not been ascribed to all of these chemical substances, but that does not necessarily mean they are without physiological effects. For example, cortisone, when first discovered, was considered to be a relatively inactive compound. It is true that it is less effective than some of the other cortical hormones regarding specific responses. It is

possible that there are other ovarian hormones to be discovered. At least it is extremely difficult to explain many physiological processes on the basis of known ovarian hormones. Parturition, for example, is a physiological process about which little is known.

We do know that calves of some breeds of dairy cattle, inheriting from both parents a single autosomal conditioning gene, are not born at the normal stage of development (Gregory, Mead and Regan, 1949-1951). Whether the presence of the gene in the heterozygous state in the mother also exerts an influence is not known, but it is known that cows carrying the gene have gestation periods of normal length if the calf does not receive the gene from both parents. No evidence is available to explain how this gene controls the time of parturition. It is most interesting that the calf must carry the gene in the homozygous state for gestation to be prolonged. The current viewpoint is that hormones, secreted by the mother, control parturition. These studies show definitely that, under certain circumstances, the fetus plays a role in determining when parturition occurs.

The point I wish to emphasize is, that we need not consider that all of the hormones are now known, and that it is merely a question of obtaining more detailed information on the physiological effects of known hormones. It is very likely that there are still unknown hormones. Therefore, it is important that we gear our thinking to the point of view that unexplored substances are present and inviting discovery.

The effect of environment upon reproductive activities

Studies initiated in John Hammond's laboratory have shown that light has an important influence upon the sexual season in the ewe (Yeates, 1948). We (unpublished) have kept ewes under constant light conditions for a period of two years during which the animals received 6 hours of daylight and 18 hours of darkness. The sexual season of these animals was not nearly as clear-cut as those kept under normal lighting conditions. Nevertheless, there still is indication of a distinct sexual season. Thus the number of daylight hours is only one of the factors involved in controlling the sexual season of the ewe.

It is probable that many factors other than light have an influence upon the reproductive activity of animals. The fact that we do not control environment sufficiently, no doubt, explains many of the aberrant results which, at the present time, defy explanation.

The role of nutrition in reproduction likewise needs further clarification. Metabolic abnormalities during lactation probably have a nutritional basis in many instances. Shaw (1947) has shown that primary ketosis in dairy cattle, a disease of lactation, can be treated successfully with cortical extracts. It has been shown, furthermore, that the disease can be successfully treated by nutritional means. The feeding of acetate (Miller and Allen, 1952) and propionate (Schultz, 1952) have been reported to have beneficial effects. Shaw et al. (1952) have compared the relative effectiveness of these compounds.

Boda and Cole (1951) gave results indicating that the prepartum level of calcium intake might have an effect upon the incidence of milk fever. Further studies (unpublished) have substantiated these early findings.

The influence of the nervous system on the reproductive processes

It has been recognized for many years that the nervous system has an influence on reproductive processes. For example, it is known that the act of mating induces ovulation in the rabbit. This is explained on the basis that the pituitary is stimulated to release gonadotrophin by nervous means. Studies by Ely and Peterson (1941) have indicated that the let-down of milk in cattle is under a neuro-hormonal control mechanism. We have recognized that certain environmental factors such as light, exert their influence by nervous pathways.

Undoubtedly, much is still to be learned regarding the influence which the nervous system has on the activity of the various endocrine glands. This is well illustrated by the recent findings concerning the control of ovulation in polyestrous animals. Though it has been recognized that ovulation in the rabbit, cat, and ferret depended upon the nervous system, it has been assumed that ovulation in spontaneously ovulating animals was solely dependent upon the endocrine system. Recent studies in the rat have indicated, however, that neurogenic stimuli are involved in the release of LH with consequent ovulation in the estrogen-treated pregnant rat and in the cyclic nonpregnant animal (Sawyer et al., 1949; Everett et al., 1949). Later studies (Everett and Sawyer, 1953) suggest that the neurogenic stimulus to be effective has a minimal duration of 20 to 35 minutes. Studies by Hansel and Trimburger (1950) indicate the involvement of a neural mechanism in the release of LH in the cow.

Conclusion

I conclude that if more rapid progress is to be made in understanding the various phases of reproduction in domestic animals, it is very important that we bring in new men who are very highly specialized in their training. For example, in the field of neuro-physiology, the field of steroid chemistry, etc. Furthermore, it is important that we do not take a defeatist attitude--that is, assume that most of the hormones have been discovered, and that all we can do is add bits of knowledge regarding their physiological effects. I feel certain that there are many hormones to be discovered. We should be constantly on the watch to uncover these substances.

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CONTENTS CONTINUED

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